Action Research for Engineering: Murine Experiments for Hypertension Research and STEM Education

by

Theo Hopper

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Biomedical Engineering)
in the University of Michigan
2023

Doctoral Committee:

Professor James Ashton-Miller, Chair
Professor Daniel Beard
Dr. Laura Hirshfield
Assistant Professor Karin Jensen
Professor David Nordsletten

Sara E. Hopper

shopper@umich.edu

ORCID iD: 0000-0002-0081-8270

Acknowledgements

There are so many people I should thank for their help and guidance. Firstly, I would like to thank my committee members. Professor James Ashton-Miller for stepping in as my chair and for all of the help over the past year. Professor Dan Beard for the experimental and physiological context for the cardiovascular components. Professor David Nordsletten for always being willing to mentor me, especially with all of the professional and career advice. It has been very comforting for me to know I have at least one true ally in academia. Thank you also for not getting mad at all of my silly little jokes. Professor Karin Jensen for also providing me with very valuable career and research advice. Thank you also for making me feel as though I had a space to belong in your lab group. Professor Laura Hirshfield for giving me the opportunity and mentorship required to take on the STEM Pride research project. I very much appreciate your willingness to start a project based on a random email with a half-fleshed out idea. Once we started talking about how much we love Target in our first meeting, I knew it would be great working with you!

I would also like to thank everyone I have had the pleasure of collaborating with. For my computational modeling work, I would like to thank Professor C. Alberto Figueroa, Professor Jay D. Humphrey, Dar Weiss, Federica Cuomo, Sara Roccabianca, Dr. Nicholas Burris, and Jacopo Ferruzzi. For my engineering education work, I would like to thank Professor Aileen Huang-Saad, Alanna Epstein, Ryan Rosario, Professor Laura Hirshfield, Jeanne Sanders, Laila Rad, and Irene Zhang. I have truly learned so much from all of you over the years, and your guidance has helped shape me into a better researcher, professional, and person.

I am also very grateful to my teaching mentors, including Professor Aileen Huang-Saad, Cameron Louttit, Melissa Wrobel, and Lizzy Mays. Thank you all for providing so much guidance and mentorship. You all went above and beyond in your roles to make sure that I had the opportunities to learn how to be a better educator. I would also like to thank everyone I have done DEI work with: Chris Tossas-Betancourt, Julianna Richie, Daniel Pfau, David Nordsletten, Irene Zhang, Elizabeth Bottorff, Rachel Patterson, Firaol Midekssa, and Laila Rad. You all brought such interesting and helpful perspectives that helped educate and motivate me for the work we did together.

The biggest thank you to everyone I worked with in the computational vascular biomechanics lab. Fede, Sabrina, and Jonas, thank you for putting up with me when I joined the lab as a force of chaos and distraction. Thank you for the mentorship and guidance you provided me with then as well. Tossas and Kritika, thank you for everything you've done for me both with lab work and personally, although you both owe me ice cream. Liz, thank you for all of the emails you've read to make me feel better before sending. Thank you to Ismael, Taeouk, Yunus, Pieter, Nitesh, Abhilash, Alex, Chris Arthurs, Ignas, Jonathan, Nic, Vasilina, and Victoria. You all truly made coming to work everyday fun. I could not have gotten to where I am without your support.

I would like to thank all of the friends I have made in Ann Arbor: Monica, Mica, Julianna, Elena, Natasha, Aarti, Haley, Lauren, Ian, Jess, Rebecca, Laila, Peter, Anthony, Evan, Paula, and Elizabeth. You are truly some of the best people I have ever met. You have made Ann Arbor feel like home to me. Your support was key for me completing my degree. Thank you for working with me for hours in random cafes, playing games, puzzling too late into the night, giving feedback on my talks, going on chaotic apple picking trips, being the best cat sitters/god parents, listening to me talk for hours about random Tiktok drama, and pretending to care about my obsessions (especially when it comes to watching Muppet movies). Thank you to Physically Present and all of my improv friends, the 3 hours a week we've dedicated for laughing was truly so important for me over the past 18 months.

Finally, I would like to thank my family for their unrelenting support: Mom, Dad, and, I guess, Kate. I always known that I am supported no matter what happens. Thank you, Mom and Kate, for the random calls about medical things or information about specific medicines. I also cannot forget to thank my true emotional support, the family pets – Riley, Dakota, Jelly Bean (Sorry, Mon and Mica, I have emotionally stolen Jelly), and Beans. Beans, you are the best first cat I could have. Thank you for all of the biscuits, cuddle time, and forcing me to leave the house and spend time outside. If you are reading this, HOW DID YOU LEARN TO READ?!?!

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

Action research is a form of research with the goal of improving standards of practice. In this thesis, action research was used to address practical issues within experimental methods for hypertension research and interventions for STEM education, including through defining the problem to be addressed, implementing a solution, assessing impacts, and providing recommendations for future improvements.

Hypertension is highly prevalent and leading contributor to death worldwide. A positive feedback loop that relates structural stiffness and hypertension, whereby stiffness increases in response to hypertension and causes further increases in blood pressure, is hypothesized to promote end-organ-damage, which contributes to the development of cardiovascular, renovascular, and neurovascular disorders. The relationship between hypertension, vascular stiffness, and organ damage is an important research focus for the potential development of therapies and diagnostic tools. This is difficult to investigate in humans due to limitations in non-invasive measurements, so murine experiments are commonly used. Action research can help maximize the clinical relevance of murine studies through optimization of experimental design. Specifically, our work investigated the impact of experimental conditions on metrics of hemodynamics and vascular structure using fluid-structure-interaction modeling of the central vasculature.

In the first aim, we investigated the similarities and differences in hemodynamics and vascular structure between young and old humans and mice. This led to insights into the appropriate use of the naturally aged mouse to study cardiovascular aging and potential targets for

development of novel mouse models. In the second aim, a computational workflow was developed to estimate awake hemodynamics and investigate the effects of anesthesia on control and hypertensive mice. We found that isoflurane anesthesia has a more profound effect on angiotensin-II infused mice than controls, which may skew the results of research studies that characterize hemodynamics with this model. Future studies should consider using alternative anesthetics or computationally adjusting results of studies with angiotensin-II infused mice under isoflurane sedation.

Computer programming is a highly sought after skill for BME graduates, but undergraduate students often struggle with knowledge of and attitudes towards the field. Action research can aid in the incorporation of research-based instructional strategies into practice, which can improve student outcomes. In the third aim, a short module was developed to improve BME undergraduate student's conceptual knowledge of and attitudes towards computer programming. Project-based learning (PBL) and scaffolding were implemented through lectures with active learning activities, labs with open-ended extra credit, and a final project. Students exhibited gains in conceptual knowledge and confidence with the material from pre- to post-course. Future improvements include expanding to a semester course, increasing the difficulty of extra credit and project, and incorporating additional connections to practice.

The climate in STEM towards members of the queer community has been previously described as chilly, which leads to lower retention and higher rates of mental health issues and closeting. In the fourth aim, action research and queer theory were applied to develop a visibility campaign, STEM Pride, to highlight the interests, achievements, and connections to the queer community of queer individuals in STEM. The impacts of the initiative were assessed, the climate was characterized, and additional needs of the community were identified through surveys and

focus groups. Future improvements include moving to more than one platform, increasing advertising efforts, hosting additional education events, highlighting the fluidity of identity, and advocating for additional safety signals in academic spaces.

Chapter 1 Introduction

1.1 Action Research

Action research is a form of research traditionally used in social sciences with the goal of improving standards of practice [1]–[4]. The basis for action research is solving an immediate problem, and it can be viewed as a framework for decision making [5], [6]. As opposed to traditional research, the insights gained from action research are directly used to inform best practices, as well as gaining general knowledge about the systems in place. This knowledge gained is subjective based on the context in which it was developed, which employs a post-modernist view of the relativity of knowledge [3], [4]. Often in action research, the researcher is also the practitioner, i.e., an educator refining their teaching strategies. Forms of action work further increase the participatory nature by including research participants in the research process [2]. Action research is a circular process, where the solution to a problem is iteratively improved upon. This is depicted in Figure 1-1, where 1) an issue in practice is observed and defined, 2) a plan is developed and implemented to address the issue, and 3) the intervention is assessed and the researcher reflects on further improvements, which starts the cycle over again [4].

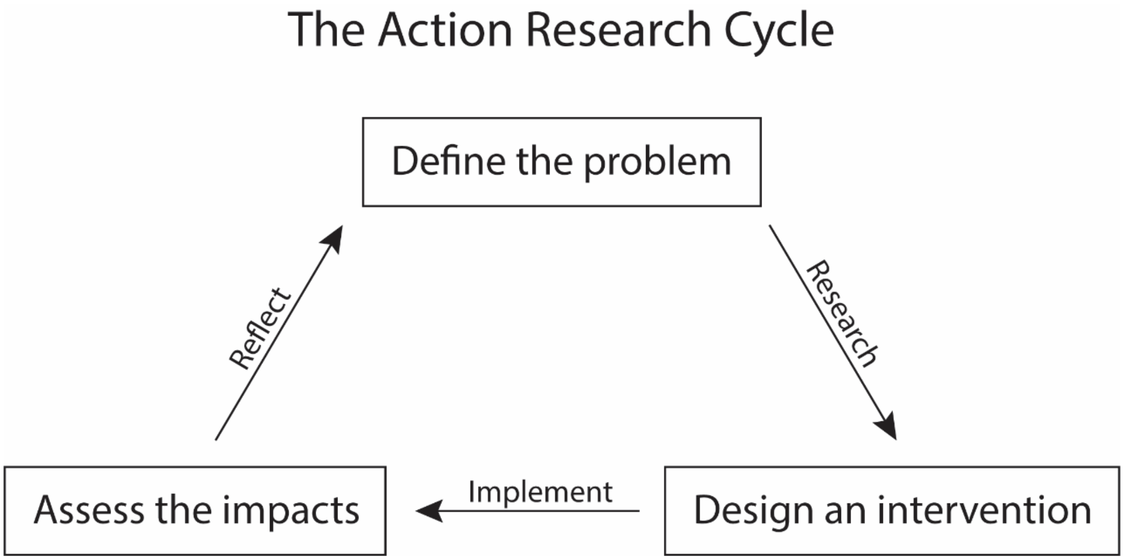


Figure 1-1. The action research cycle, adapted from [3], [7].

To better understand the process of action research, consider a professor who wants to improve upon their practice in the classroom. This professor starts by characterizing the classroom environment and identifying issues that need to be addressed. They observe their students and search in literature for strategies employed by others to resolve the issue. The professor will then make an informed change to their teaching practices. The effects of this will be assessed, and the professor reflects on the adaptation, which leads to the identification of further issues or areas of improvement. This process of iteratively improving their teaching strategies continues, which not only gives insights into best-practices but also gathers knowledge about the issue at hand. Contrary to traditional research practices, critical personal reflection is a key process in action research [1]. Because the researcher is an active participant (i.e., an insider) in the practice, this personal reflection leads to further identification of problems associated with intervention to which someone who does not engage in the practice (i.e., an outsider) may be blind [2]. For example, an educational theorist may present a solution to a professor's problem that is not feasible in practice because of large class sizes or limited time with students. In action research, the researcher is both the theorizer and practitioner, closing the gap between theory and practice [2].

Different forms of action research have been defined based on their key goals, which include technical, practical, and critical [2]. The goal of technical action research is to improve the outcomes of practice, like in an experimenter improving their research procedures to ensure generalizable knowledge [7]. Technical action research has been applied previously in engineering design [8]–[11]. Practical action research focuses on improving the processes involved in practice, like a professor improving their teaching strategies or a medical professional improving their interventions with patients [2]. Practical action research has been used in engineering education [12]–[14] and in industry engineering processes [6], [11], [15]. Critical action research uses critical theory and action research to promote change to oppressive systems [2], [4]. Identifying the negative societal forces on an individual is key in critical action research. For example, a professor that uses critical action research to improve inclusivity would analyze and create interventions to address the societal and historical factors that lead to the marginalization of groups of students in their classroom [2]. Critical action research has been used, albeit not frequently, in engineering education research to promote inclusivity [16].

1.2 Project Scope

In this work, all three described types of action research were applied to the field of engineering. First, technical action research was used to improve the generalizability of murine models for hypertension research to human disease. Computer modeling approaches were used to analyze current experimental strategies for murine studies of hypertension. Secondly, practical action research was used to improve the teaching practices for computer programming skills for biomedical engineering students. Finally, critical action research was used to improve the climate of STEM education for LGBTQIA+ students. For each of these applications, this work describes the stages in the action research cycle that were addressed, including defining the problem, implementation of a plan, and reflection, and makes recommendations for future iterations.

This thesis is structured as follows:

Chapters 2-5: Technical Action Research for Murine Models of Hypertension

Chapter 2: Introduction to Hypertension Research. Discussion of hypertension pathophysiology, limitations of experimental methods for studying *in vivo* disease conditions, considerations for use of murine models, and background of fluid-solid-interaction (FSI) modeling for the cardiovascular system.

Chapter 3: Generalizability of Results for Studying Cardiovascular Aging: Analysis of Naturally Aged Mouse Model. Computational models of young and old humans and mice were built and calibrated from experimental and population-based data. The effects of aging on regional and global hemodynamics and vascular structure were examined to determine species-related differences.

Chapter 4: Generalizability of Results for Studying Hypertension: Impact of Isoflurane Anesthesia with Angiotensin-II Infused Mice. A computational workflow was developed to estimate the awake conditions of and account for the differential effects of isoflurane anesthesia on angiotensin II-infused mice. Awake computational models were compared to analyze the impact of angiotensin II-infusion on aortic structure and hemodynamics.

Chapter 5: Reflection and Recommendation for Changes to Experimental Practice

Chapters 6-9: Action Research for STEM Education

Chapter 6: Introduction to Interventions for STEM Education. Background of social-psychological theory, student-centered STEM education, climate in STEM for underrepresented groups, and discussion of research-based educational strategies.

Chapter 7: Instructional Practices for Teaching Computer Programming: Applying Research-based Teaching Strategies. A 3-week, 1-credit short course was developed incorporating project-based learning and scaffolding to increase student perceptions of, confidence in, and conceptual understanding of computer programming. The effects of the educational strategies were assessed using concept maps and surveys.

Chapter 8: Climate in STEM for LGBTQIA+ Individuals: Development of a Visibility Campaign. A social media initiative to increase visibility and awareness was created to improve the climate of LGBTQIA+ individuals in STEM. The effects of this initiative on participants were assessed through a survey and focus groups.

Chapter 9: Reflection and Recommendation for Changes to STEM Education Practice

Chapter 10: Conclusions

Chapter 2 Introduction to Hypertension Research

2.1 Motivation

Hypertension is defined as consistently elevated blood pressure, specifically when systolic blood pressure is greater than 130 mmHg or diastolic blood pressure is greater than 80 mmHg [17]. Factors that contribute to the development of hypertension include poor lifestyle, including high sodium diet, lack of exercise, and tobacco and alcohol use; genetic factors; and other conditions, such as diabetes, kidney disease, renal artery stenosis, sleep apnea, and autoimmune diseases [18], [19]. One-hundred and sixteen million people, nearly half of the adult population in the United States, have diagnosed hypertension [17]. Furthermore, hypertension is a precursor of organ damage, including in the heart, brain, and kidneys, and other cardiovascular diseases (CVD), including heart disease, heart failure, and stroke [20], [21]. Because of these, hypertension is a leading contributor to death worldwide, including to 670,000 deaths in the United States in 2020 [17].

There is a documented positive feedback relationship between hypertension and arterial stiffening that may be a root cause of the cardiovascular sequelae accompanying hypertension [22], [23]. There is a need to investigate the structural vascular changes associated with increasing blood pressure and further alterations in hemodynamics that contribute to organ damage. Such information may lead to advances in clinical diagnoses, stratification, and treatment strategies. However, it is difficult to obtain high resolution and high accuracy measurements for hemodynamics and arterial stiffening *in vivo* with clinical metrics from human subjects. Animal models, specifically murine models, give researchers the necessary spatial and time resolution through the use of invasive and *ex vivo* techniques.

Despite the experimental advantages of murine models, the differences between humans and mice may limit their ability to be used to gather information about the human disease state [24]. Careful experimental design must be used to ensure that results from murine experiments are generalizable. Technical action research can be used to optimize the experimental design practices to ensure this generalizability. In this work, the impact of experimental design decisions (i.e., the field "solutions" for studying hypertension in mice) on resulting hemodynamics are analyzed using computational modeling, leading to recommendations for best practices for murine experiments. Fluid-solid-interaction (FSI) models of the cardiovascular system were built from the results of experimental murine to explore *in vivo* hemodynamic and structural effects of hypertension and the impact of experimental decisions on the applicability of results to the human disease state. In this chapter, I will define the issue being addressed via action research and discuss the methods used to analyze current solutions. Below you will find a discussion of hypertension pathophysiology and vascular stiffness, clinical metrics for examining blood pressure and vascular stiffness, considerations for murine hypertension experiments, and computational techniques.

2.2 Hypertension and Vascular Stiffness

Progression of hypertension and its contribution to other CVD is hypothesized to be caused by an insidious positive feedback relationship with increased stiffness of the central vasculature (Figure 2-1), in which hypertension is both a cause of and contributor to this increase in stiffness [22]. Briefly, an increase in blood pressure is accompanied by an increase in circumferential stress on the vascular wall. There is a homeostatic response to mitigate this stress with an increase in vascular thickness, which stiffens the vascular wall [22], [23]. Increased stiffness affects global hemodynamics reflecting pressure waves earlier in the cardiac cycle, which augments systolic blood pressure (Figure 2-2) [21], [23], [25]. Further, increases in structural stiffness reduce the sensitivity of baroreceptors, thereby diminishing the ability to maintain homeostatic control of blood pressure [21]. Vessel stiffness can be described in two ways: material stiffness, which is the intrinsic properties of its components, and structural stiffness, which considers the vessel thickness along with material stiffness and is indicative of these alteration in hemodynamics [26].

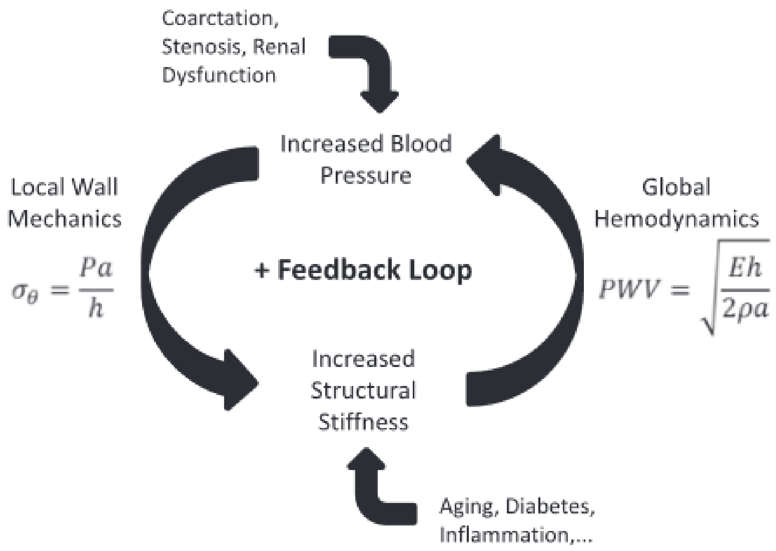


Figure 2-1. Insidious positive loop between structural stiffness and blood pressure from [22].

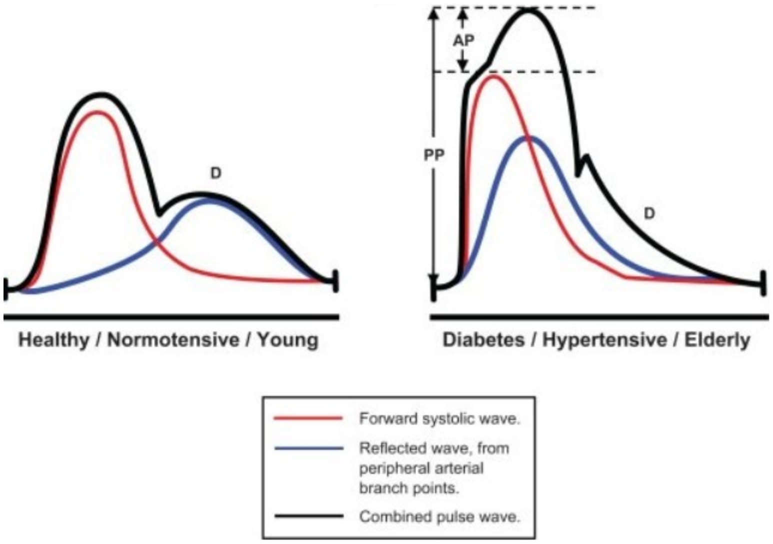


Figure 2-2. Reflective waves reach the aortic inlet earlier in the cardiac cycle with stiffer vessel from [27].

Vascular stiffening has been observed to precede systemic hypertension, typically in response to conditions that alter the molecular structure of the arterial wall, including aging, connective tissue disorders, and increased inflammation [28]. The material stiffness of a vessel is dependent on the content and organization of its molecular components, which vary based on type of vessel and location relative to the heart. The main molecules that contribute to passive vascular material stiffness are elastin and collagen, which give vessels a nonlinear stress-strain relationship, as seen in Figure 2-3 [29]–[31]. Elastin is responsible for the elasticity of the vessel wall, seen more at low strains, while collagen is primarily responsible for the material strength and load bearing capacity, seen more at higher strains [29]. Collagen fiber arrangement, content, and degree of crosslinking has a large effect on vascular stiffness [30]. Collagen fibers are arranged into families based on directionally in the arterial wall, including circumferentially, axially, and diagonally, which causes the arterial wall to be anisotropic [26], [30], [32], [33]. The content and organization of elastin and collagen varies along the length of the aorta, causing regionally varying tissue properties [34], [35]. Elastic arteries (as opposed to muscular arteries) are more proximal to the heart and have a higher ratio of elastin to collagen fibers [29]. The elastic properties of which are important for maintaining healthy hemodynamics, especially in the ascending thoracic aorta (ATA). The ATA expands during systole in response to the increase in blood volume, and the elastic properties cause it to recoil during diastole, which aids in distal blood flow and dampens the pulse pressure heading to peripheral capillary beds [21], [29].

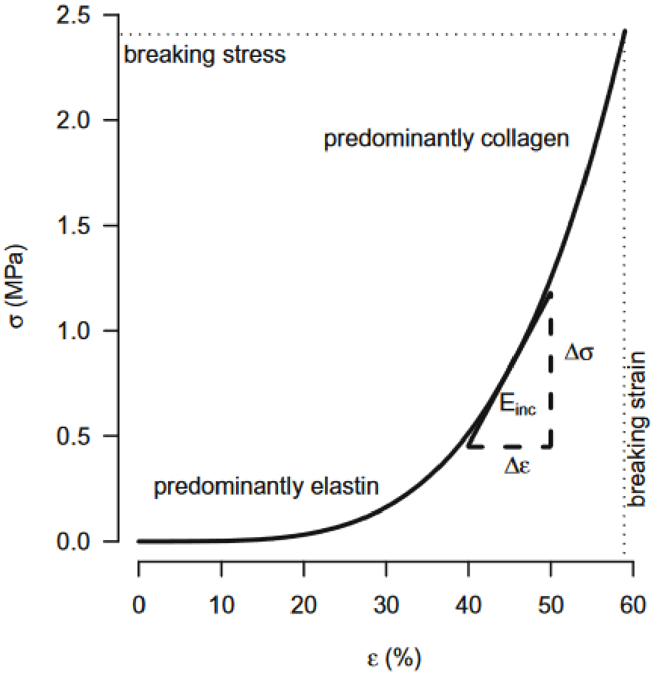


Figure 2-3. Non-linear stress-strain relationship of vascular tissue with contributions of elastin and collagen labeled from [31].

Arterial stiffening is caused by growth and remodeling of the arterial wall. Collagen fibers are constantly degraded and deposited in the arterial wall based on mechanosensing of vascular cells [29], [30]. With an increase in stress associated with hypertension, more collagen fibers are deposited than degraded in the wall, causing an increase in collagen content. In addition to collagen content, hypertension also alters collagen organization and crosslinking, which also impacts material stiffness [36]. In contrast to collagen fibers, the majority of elastin fibers are synthesized during gestational growth and early childhood. In normotensive conditions, elastin fibers are durable and maintain their integrity in the vessel wall. However, the half-life of elastin is around

40 years [37], so elastin starts to degrade with age. Furthermore, increased cyclic load, e.g. with hypertension, can lead to elastin fragmentation and degradation [38]. Elastin fiber degradation decreases the elastic potential of the arterial wall, but the fibers are also replaced with collagen, which further increases material stiffness [39]. These instances of vessel remodeling often promote vessel thickening, causing further increases in structural stiffness.

Alterations in hemodynamics from increased structural stiffness cause end-organ damage and further pathology, contributing to hypertension-related mortality [21]. With vascular stiffening, the expanding and recoiling of the ATA is impaired, and peripheral vasculature is exposed to higher pulse pressures, damaging the capillary beds of low resistance organs, mainly the brain and kidneys [21]. Changes in wave reflection with vascular stiffening discussed earlier (Figure 2-2) promote cardiac damage. Subendocardial perfusion occurs predominantly in the diastolic phase of the cardiac cycle; in the healthy vasculature, pressure wave reflections reach the aortic inlet during early diastole, which increases pressure and drives coronary perfusion. When wave reflections arrive earlier in the cardiac cycle due to stiffening, the perfusion pressure gradient that drives coronary artery filling is decreased and cardiac afterload is increased [23]. High afterload also increases cardiac work and left ventricular stress, potentially leading to hypertrophy of the left ventricle and increasing metabolic demand of the myocardium [25], [39], [40].

2.3 Clinical Measurements for Hypertension and Vascular Stiffness

Given the relationship between vascular stiffness, hemodynamics, and cardiovascular mortality, there is a need for experimental studies that relate *in vivo* blood pressure and vascular stiffness. In humans, metrics related to aortic pressure and stiffness are difficult to obtain, either requiring invasive procedures or applying assumptions to use indirect, non-invasive measurements. Furthermore, these measurements often do not have the time or spatial resolution necessary to gather key insights pertaining to progression of hypertension or the effects of vascular stiffening.

In terms of pressure, invasive catheterization is necessary to directly obtain central aortic pressure waveforms. This is typically not performed on healthy human subjects because of discomfort and risk, so non-invasive measurement techniques are often used on peripheral arteries. Such techniques include brachial artery cuffs, applanation tonometry, and ultrasound [41]. Clinically, systolic and diastolic blood pressure values are obtained from the brachial artery using a sphygmomanometer (cuff). Both applanation tonometry and ultrasound techniques utilize the movement of the arterial wall and blood pressure cuff measurements to estimate peripheral pressure waveforms [41]. No matter the non-invasive method, pressure is measured from the peripheral artery and assumed to correlate to the central arterial pressure. However, pulse pressure increases distally from the aortic inlet in a manner dependent on the structural properties of the aorta (Figure 2-4), so pulse pressure will be higher in the peripheral arteries and the difference will be smaller in stiffer arteries [42]. The FDA approved Sphygmocor applies a transfer function to estimate the central blood pressure waveform from the brachial artery pressure waveforms obtained via tonometry [43]. Schultz *et al*. (2020) found that the Sphygmocor underestimated systolic pressure compared to invasive measurements, and the difference was larger with higher systolic pressure [44]. Invasive catheter measurements are currently the only way to guarantee accurate central arterial pressure in all subjects.

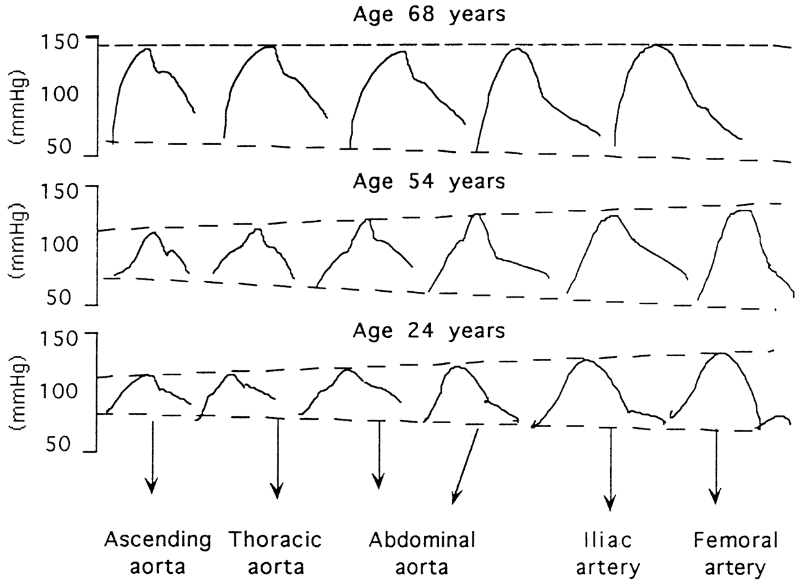


Figure 2-4. Pulse amplification down the aorta. The magnitude of which decreases with increased age and structural stiffness from [45].

Aortic tissue properties can only truly be captured through material testing of excised vascular tissue. However, even with such mechanical testing, the *in vivo* mechanical properties need to be interpreted from *ex vivo* measurements. *In vivo* structural stiffness can be estimated clinically from human subjects using available metrics, such as pressure and vessel diameter. Such clinical metrics include pulse wave velocity (PWV), augmentation index (AIx), and relationships between pressure load and vessel diameter, like distensibility or compliance [38], [46]. While these metrics have been previously shown to correlate to cardiovascular disease risk [39], their use as research tools for understanding the relationship between hypertension and structural alterations is limited by an inability to capture regional variations in stiffness and large operator variability.

PWV is the current gold standard for the clinical assessment of stiffness and is based on the guiding principle that pulse waves travel faster in stiffer arteries [42]. Clinical PWV is assessed from speed of traveling pulse waves between two points in the vasculature, typically at the carotid and femoral arteries (cf-PWV) [46], [47]. cf-PWV is clinically measured by calculating the pulse transit time, estimated by the difference in times of the systolic upstroke of the cardiac cycle from pressure or volume waveforms, and the distance between measurement points, as seen in Figure 2-5 [47]. PWV is related to vessel stiffness (E) through the Moens-Korteweg equation [47], seen in Equation 2-1. PWV has been previously found to correlate well with cardiovascular events and mortality [39]. Despite this, the efficacy of PWV as a tool for investigating aortic stiffening with hypertension has been previously examined [48]–[50]. Firstly, in the Moen-Korteweg equation, parameters such as vessel thickness and radius need to averaged over the length of the arterial system. Secondly, the distance used to clinically assess PWV is based on the measurement position on the skin and not aortic length [49]. This is especially problematic in older subjects, as the aorta becomes more tortuous with age [51]. Thirdly, not only does PWV not capture regional variations in vessel stiffness, previous studies have found that PWV underestimates aortic stiffness with larger regional variations [48]. Finally, there is high operator variability, especially when pressure waveforms are used to estimate transit time [47].

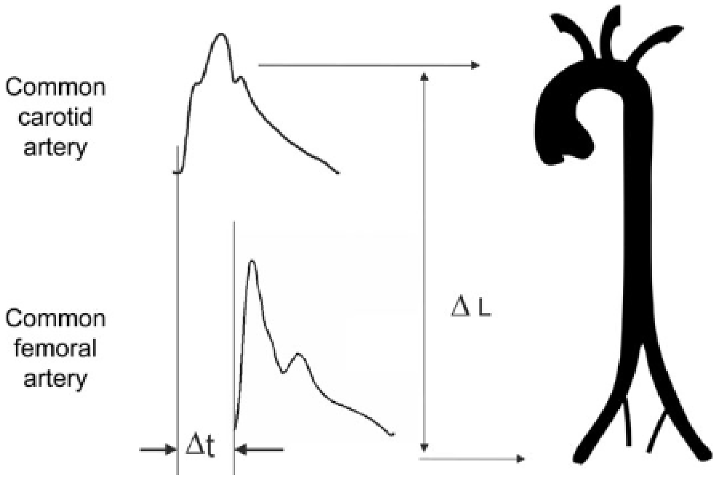


Figure 2-5. Clinical assessment of cf-PWV involves measurements of pressure transit time (Δt) and length (ΔL) from [46]

$$PWV = \frac{\Delta L}{\Delta t} = \sqrt{Eh/2\rho a}$$

Equation 2-1. Relation of clinical estimation of PWV to vessel stiffness via the Moens-Korteweg equation [22]. E is vessel material stiffness, h is vessel thickness, ρ is blood density, and a is vessel inner radius.

AIx uses the principles of backward wave reflections to the aortic root as a metric for average aortic stiffness [46]. As described previously, backward wave reflections arrive at the base of the aorta earlier in the cardiac cycle in a stiffer vessel, which augments systolic pressure [25]. AIx is calculated as the amplitude of this reflected wave, estimated by the difference in systolic peak pressures after peak blood flow (Figure 2-6) and known as augmentation pressure (AG) , divided by pulse pressure [31], [46]. Clinical determination of AIx requires analysis of the central pressure waveform, which, as discussed previously, is difficult to obtain. Non-invasive determination of AIx rely on tonometry measurements of a peripheral artery with a transfer function to account for pulse pressure amplification, like with the Sphygmocor device [52]. Invasive pressures from catheterization can be used to more directly calculate AIx. Previous studies have found that factors not related to arterial stiffness can affect AIx values, including heart rate [53] and LV myocardial shortening [52]. Studies also propose that AG may be a better indicator of arterial stiffness than AIx [52], [54].

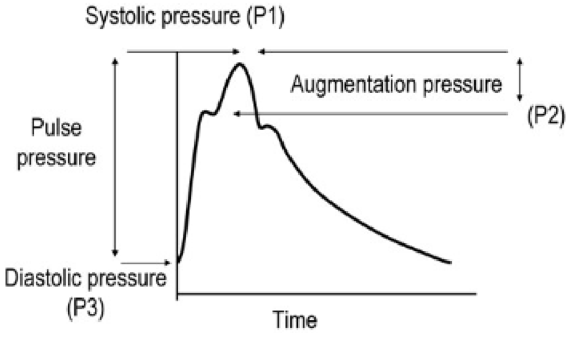


Figure 2-6. Augmentation pressure is the pressure increase from reflective waves from [46].

Multiple clinical metrics relate the load, blood pressure, to the deformations, changes in vessel diameter or area, including distensibility (D), compliance (C), Peterson's Modulus (E_p), and stiffness index (β) [31]. D and C estimate the ability of the vasculature to respond to increased blood load, so they are inverse metrics for vessel stiffness (E_p and β) [31]. The relationships used to calculate these metrics can be seen in Table 2-1. As opposed to PWV and AIx, these measure local vessel properties and are not reflective of global aortic stiffness. Furthermore, due to the limited ability to measure central pressure non-invasively, these metrics are often taken from peripheral arteries, where pressure can be measured via tonometry or cuff and vessel diameter can be measured via Doppler Ultrasound [55]. Aortic stiffness can be estimated through these metrics with diameter measurements of the aortic region of interest and peripheral pressure measurements by negating the effects of pressure amplification; however, given the alterations in this amplification with age, this may not be an effective strategy for determining the effects of stiffening with age or hypertension[42].

Table 2-1. Pressure and diameter based metrics for vascular stiffness, adapted from [31]. d_{sys} *and* d_{dias} *are systolic and diastolic diameter, respectively.* P_{sys} *and* P_{dias} *are systolic and diameter, respectively. *Distensibility and compliance can be estimated based on diameter or area change, based on imaging modality and resolution [26].*

Clinical Metric	Relationship
Distensibility	$D = \frac{d_{sys} - d_{dias}}{d_{dias}(P_{sys} - P_{dias})} *$
Compliance	$C = \frac{d_{sys} - d_{dias}}{P_{sys} - P_{dias}} *$
Peterson's Modulus	$E_p = \frac{(P_{sys} - P_{dias})d_{dias}}{d_{sys} - d_{sys}} = \frac{1}{D}$
Stiffness Index	$\beta = \frac{\ln\left(\frac{P_{sys}}{P_{dias}}\right)}{(d_{sys} - d_{dias})/d_{dias}}$

2.4 Murine Experiments for Hypertension Research

Due to difficulties in measuring central blood pressure and arterial stiffness in human subjects, animal models are often used. Furthermore, animal models increase experimental control, allowing researchers to independently examine factors related to hypertension and aortic stiffening without comorbidities or noise related to human subjects. Murine experiments are often used for cardiovascular disease research, as mice are inexpensive; have short, well-characterized life-spans;

and are relatively easy to manipulate genetically [26], [56]. Various factors impact the applicability of murine studies to human disease, including the disease model, background, and environmental conditions.

Numerous murine models exist to study hypertension based on genetic manipulation and surgical or pharmacological intervention [57]. Common genetic murine models include the spontaneous hypertensive mouse (*BPH/2Slg*) and knockouts of genes relating to homeostatic control of blood pressure, including renin-aldosterone-angiotensin system (RAAS) and nitrous oxide (NO) synthesis [58]. Surgical induction of hypertension includes banding of the aorta or renal artery to increase resistance to blood flow [56]. Pharmacological induction of hypertension includes manipulation of blood pressure control, also through RAAS or NO synthesis, or increasing the amount of salt or fat content [56], [57]. Known differences between humans and mice can be used to develop disease models that more closely mimic the human condition. For example, given the half-life of elastin is around forty years [37] and the mouse lifespan is around 2-3 years [59], elastin degradation does not contribute to natural mouse vascular aging. Based on this, aortic aging models have been developed that incorporate loss of elastin structural integrity, including infusion of elastases [60] and modification of genes related to elastin synthesis or structure, including elastin and fibulin-5 [32], [48], [61]. No matter the specific mechanism, these models rely on utilizing well-characterized causes of hypertension and, therefore, intrinsically cannot capture the clinical incidences of hypertension with unknown causes; furthermore, clinical presentation of hypertension is often combined with other cardiovascular risk factors, like diabetes and obesity, which is not taken into account with single phenotype mouse models [57].

In addition to the disease model, researchers need to consider the genetic strain, sex, and age of mice used. There are multiple inbred mouse strains (*C57Bl6/N*, *129SvPAS*, etc.) commonly used in research, in addition to mixed breeds of these [58]. Previous studies have found that normotensive blood pressure differs between mouse strain independent of other environmental factors [58], [62]. Similar to humans, cardiovascular phenotypes in mice show sex-based dimorphisms, including in the presentation and effects of hypertension [56]–[58]. In addition to analyzing differences between binary sexes, methods of blocking hormones, removing gonads, and intersex mouse models can be used to examine the role of sex-based characteristics in cardiovascular phenotypes [56]. The mouse lifespan has been extensively studied, characterized in terms of development stages, and related to the human lifecycle [59]. For example, it is known that a 2 year old *C57Bl6/N* mouse is approximately equivalent to a 69 year old human, which can be used when examining the effects of aging on cardiovascular physiology [59].

Environmental conditions can have a large impact on the results of cardiovascular studies. Of note, increasing frequency of handling for mice can reduce the amount of stress experienced during data collection [56]. This is especially important during awake blood pressure monitoring via tail blood pressure cuff, as increased stress can lead to overestimating resting blood pressure and heart rate [63]. Data collection requiring long measurement times; stillness of the animal, for example during imaging; discomfort; or invasive procedures require the application of anesthesia [64]. Choice and level of anesthetic can lead to inconsistencies in results [64], as can variations in temperature or physical conditions during data collection [56]. To improve generalizability of results from these studies, technical action research can be used to optimize experimental conditions.

2.5 Computational Modeling Methodology

Computational blood flow modeling has emerged as an important tool to examine cardiovascular disease, as it allows researchers to analyze *in vivo* hemodynamics with higher resolution than is feasible in experimental or clinical settings. FSI modeling involves the coupling of arterial wall motion with blood flow, which is necessary to examine the effects of changes in arterial structure on hemodynamics [65]. Other computational methods use a rigid-wall formulation, which reduces the computational time, complexity, and data collection necessary, but at the cost of accuracy to *in vivo* conditions and limited analysis potential [66]. In this work, we utilized the open-source finite-element modeling (FEM) software CRIMSON to create FSI models of the cardiovascular system consisting of a 3D domain containing the aorta and main branches coupled to reduced order Windkessel models at the outlets [66]–[68]. The CRIMSON software uses a coupled momentum model (CMM) to solve the weak form of the incompressible Navier-Stokes equations for blood flow with a linear membrane model for the arterial wall [66], [67].

To build computational models, the 3D domain is manually segmented from medical images and is discretized into tetrahedral element meshes. Boundary conditions must be applied to this domain to represent the inflow of blood, tissue properties, and truncated distal vasculature. Application of such boundary conditions should be dependent on the goals of computational analysis. A pulsatile flow waveform reconstructed from Doppler ultrasound or phase contrast magnetic resonance imaging (PC-MRI) can inform the inflow boundary condition. Reduced order heart models can also be utilized at the inlet to gain additional information pertaining to cardiac function; however, these require additional measurements, assumptions, and iterative tuning pertaining to left ventricular function [69], [70]. In this work, we are primarily interested in studying the vasculature system, so an inflow waveform was imposed at the aortic root.

Numerous constitutive relations have been established to model the biomechanical properties of the vasculature with various complexity. The simplest approximation assumes a linear stress-strain relationship, which allows stiffness to be captured with a single constant, the

Young's Modulus (E). E is the ratio of stress applied by a specified load, in this case blood pressure, to the strain experienced by the vessel, in this case diameter or area change. E can be estimated from clinical metrics discussed in Clinical Measurements for Hypertension and Vascular Stiffness, for example with PWV and the Moens-Korteweg equation. However, as discussed previously, arterial tissue has known non-linear behavior [29]. Because we are interested in looking at arterial stiffening in depth, we used the four-fiber family model as the constitutive model for vascular tissue properties. The four-fiber family model incorporates the effects of four different collagen fiber orientations: circumferential, axial, and two diagonal angles [26], [71]. The strain energy density function (W) for the four-fiber family model can be seen in Equation 2-2. The parameters of W ($\mathrm{c}, \mathrm{c}_1^{\mathrm{i}}, \mathrm{c}_2^{\mathrm{i}}, \alpha_0$) are fit for a region of vascular tissue based on the results of biaxial tissue testing [26]. The theory of small deformations superimposed on large can then be used to linearize the non-linear estimation around *in vivo* circumferential and axial stretch values to obtain an anisotropic, 5x5 stiffness matrix and thickness value [71]. Regionally varying tissue properties are applied to the aorta and main branches, which allows us to investigate the effects of regional differences in stiffening on global and local hemodynamics. A traction boundary was also applied to the vascular walls to incorporate perivascular support, which is modeled with stiffness ($\mathrm{k_s}$) and damping ($\mathrm{c_s}$) coefficients [34], [72], [73]. Values of these coefficients are iteratively tuned to match experimental data.

$$W(\boldsymbol{C}, \boldsymbol{M}^{\boldsymbol{i}}) = \frac{c}{2}(I_C - 3) + \sum_{i=1}^{4} \frac{c_1^i}{4c_2^i}\{\exp[c_2^i(I_4^i - 1)^2] - 1\}$$

Equation 2-2. Strain energy density (W) function for the four-fiber family constitutive model. c, c_1^i, *and* c_2^i *are material parameters,* I_C *is the first invariant of the right Cauchy-Green tensor,* I_4^i *represents the square of the stretch and depends on the fiber orientation,* α_0^i, *where* $\alpha_0^1 = 0°$ *(axial),* $\alpha_0^2 = 90°$ *(circumferential), and* $\alpha_0^3 = -\alpha_0^4 = \alpha_0$ *(diagonal) from [74].*

Multiple methods have been established to apply outflow boundary conditions, which also range in computational complexity. The simplest and least accurate to *in vivo* conditions is to apply a Dirichlet pressure boundary [68]. To better account for and characterized the vasculature truncated from the model, we apply a three-element Windkessel model to the outlets, which models the distal vasculature using a circuit analog composed of proximal (R_p) and distal (R_d) resistances and compliance (C) [68], [75]. The values for these parameters are initially estimated from experimental and population-based hemodynamic measurements [76], and then iteratively adjusted to match experimental hemodynamic data that is not used to inform the model, including arterial pressure and addition flows measured from locations other than the aortic inlet [48].

Chapter 3 Generalizability of Results for Studying Cardiovascular Aging: Analysis of Naturally Aged Mouse Model

This chapter has been adapted from a peer reviewed version of this work:

Hopper SE, Cuomo F, Ferruzzi J, Burris NS, Roccabianca S, Humphrey JD and Figueroa CA (2021) Comparative Study of Human and Murine Aortic Biomechanics and Hemodynamics in Vascular Aging. *Front. Physiol.* 12:746796. doi: 10.3389/fphys.2021.746796

3.1 Introduction

Age is a primary risk factor for numerous cardiovascular diseases, including hypertension and associated secondary diseases [77], [78]. Even in the absence of co-morbidities that often arise in aging, natural aging-induced changes in structure and function of the vascular system include pronounced central artery stiffening [22], [25]. Such structural stiffening results in large part from changes in the composition and thickness of the arterial wall: elastin fibers undergo fatigue-related damage and can begin to degrade naturally despite their long half-life, while proteoglycans and collagen fibers can both remodel and accumulate, also influenced by increased cross-linking [79]. Whereas loss of elastic fiber integrity can reduce central artery resilience, and thus overall biomechanical functionality, the overall increase in structural stiffness due to accumulating extracellular matrix impacts hemodynamics as discussed in Chapter 2, leading to increases in central systolic pressure and a deeper penetration of pulsatility into the peripheral vessels that affects end-organ function [21], [25], [39].

Due to limitations in human clinical studies discussed previously, mouse models are often used to study vascular aging. When performing these studies, choice of mouse model is particularly important for ensuring results are applicable for human disease research. Obvious differences between humans and mice include their very different lifespans (70+ years versus 2+ years [24]) and body size, with adult mice weighing approximately 0.05% that of the adult human [80]. Cardiovascular differences include cardiac output (5 L/min for humans [81], 15 mL/min for mice [80]), which reflects the size difference, but also heart rate (60 bpm for humans, 600 bpm for mice [82]). Blood pressure is similar between the species. Notwithstanding the order of magnitude higher heart rate in mice, the expected number of cardiac cycles over a lifetime is still higher in humans (2.2×10^9) than in mice (6.3×10^8), which is expected to impact mechanical fatigue-induced loss of elastic fiber integrity [79]. Indeed, given that the normal half-life of vascular elastin is many decades [31], [83], it appears that arterial aging in mice can be attributed more to the accumulation of proteoglycans and remodeled collagen [84], [85] than to the loss of elastic fiber integrity that occurs in humans alongside matrix remodeling. Further differences between human and murine vascular aging remain to be established.

Action research can be used to improve murine models for cardiovascular aging research, which can be seen in Figure 3-1. The issue that is addressed is the lack of generalizability of results from murine models for aging to humans. Based on this issue, we designed a study to compare human and murine natural aortic aging on hemodynamics and vascular structure with computational modeling, the results of which can be used to inform the use of murine models for future studies. In this study, FSI models were built for young and old female adult humans and mice to compare hemodynamic and biomechanical effects of aging for the species. For humans, representative computational models for young and old adult subjects were defined using imaging data, non-invasive pressure measurements, and population-specific arterial wall properties. For the mice, representative population-based computational models for young and old adults were obtained using *in vivo* and *in vitro* data on vascular anatomy, hemodynamics, and wall mechanics. Due to the differences in human and mouse data collection, special care was taken to ensure that the computational models were informed consistently between the species. Both human and mouse subjects were considered healthy, with no comorbidities, with the ages between species generally equivalent. Structural and hemodynamic results were compared to determine the species- and age-related differences between subjects. The reflection and recommendations from the results of this study can be found in Chapter 5. Future research should implement the recommendations based on the differences identified here and perform future iterations of the action research cycle.

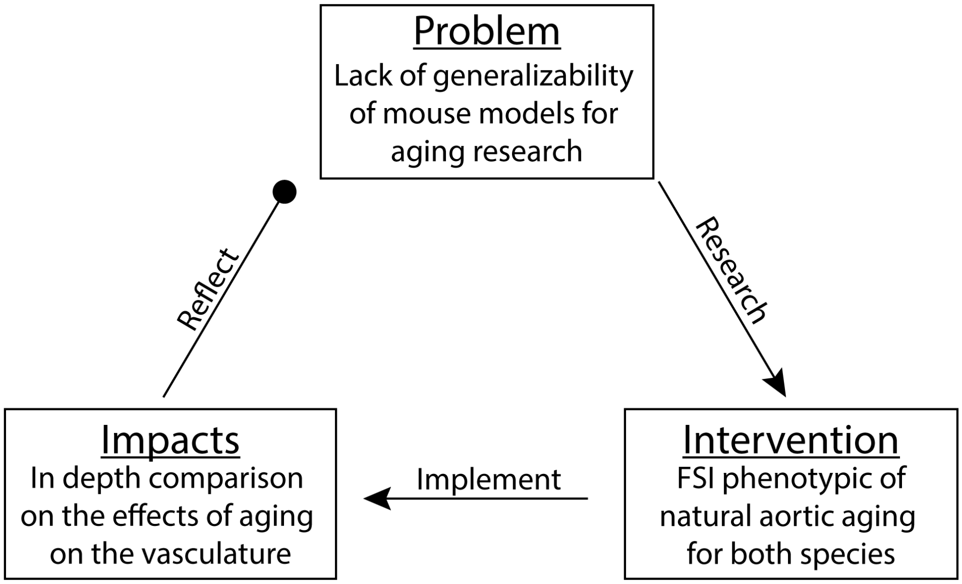


Figure 3-1. Action research cycle to improve the lack of generalizability of mouse models for vascular aging research. In this study, we stop after the reflection point with recommendations based on this first iteration of the cycle. Future studies should continue this work with further iterations.

3.2 Methods

In setting an age correspondence between mice and humans, the Jax Mice website (jax.org) suggests that a 13-27 week old mouse is similar to a 20-30 year old human, and an 81-108 week old mouse is similar to a 56-69 year old human [59]. Imaging and brachial cuff pressure data were obtained for young adult (31 year old) and older adult (81 year old) female human subjects. This study was approved by the University of Michigan Board of Review (HUM00041514). *In vivo* imaging and *ex vivo* biomechanical testing data were obtained for young adult (20 week old) and older adult (100 week old) female mice. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Yale University. The mice had a mixed *C57BL/6* x *129/SvEv* background, generated as *Fbln5*$^{+/+}$ by breeding *Fbln5*$^{+/-}$ heterozygous pairs [86]. For the young adult mice, data were collected for three cohorts at 20 weeks of age: one for anatomy, one for hemodynamics, and one for wall mechanics. For the old adult mice, data were collected from two cohorts that aged naturally to 100 weeks: one for anatomy and one for both hemodynamics and wall mechanics.

3.2.1 Experimental

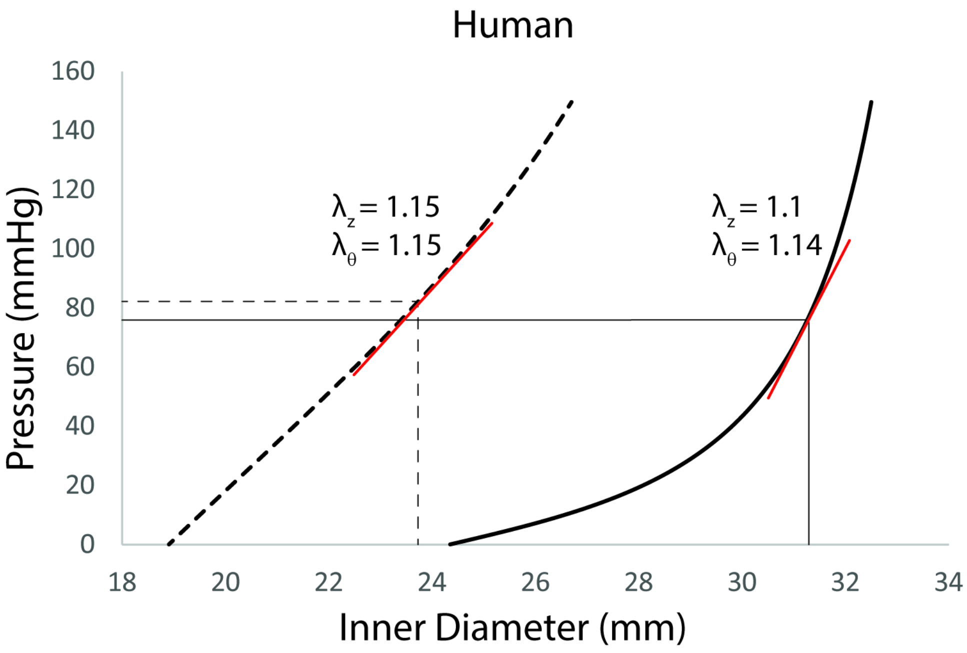
Vascular Anatomy. Magnetic resonance angiography (MRA) was performed on human subjects at the University of Michigan Medical Center. These exams were performed on 3T MRI scanners (Ingenia, Philips, Best, The Netherlands) using a 32-channel torso coil. A non-contrast MRA was performed spanning the thoraco-abdominal aorta using a 3D balanced turbo field echo sequence with navigator-based respiratory compensation (TE: 1.3 ms, TR: 4.3 ms, resolution: 0.7 x 0.7 x 1.5 mm).

As described previously [48], mice were anesthetized with 1-2% isoflurane and given a bolus intravenous (jugular vein) injection of nanoemulsion formulation (Fenestra VC, MediLumine Inc., Montreal, CA), at a dose of 0.2 ml/20 g, as a blood-pool contrast agent for prolonged vascular imaging. The animal was immediately placed prone in a micro-CT scanner (eXplore CT120, GE healthcare) for non-gated whole-body scanning. Images were reconstructed as isotropic 49 x 49 x 49 μm^3 voxels. A relatively constant heart rate (± 10%) was achieved by careful maintenance of isoflurane anesthesia and body temperature.

Hemodynamics. 40-phase, 2D phase-contrast MRI (PC-MRI) images were acquired in planes orthogonal to the human aorta at the levels of the mid-ascending aorta and distal descending aorta at the diaphragm to obtain information concerning blood velocity and vessel area over the cardiac cycle (TE: 3.0 ms, TR: 5.0 ms, slice thickness: 8 mm, temporal resolution: 19 ms, velocity encoding value: 150 cm/sec). Brachial artery blood pressure was measured with a standard non-invasive cuff method, averaged from patient data dating back two years (averaged values of 111/66 mmHg for young adult and 111/55 mmHg for old adult). It is important to note that the older human was considered healthy and free of cardiovascular diseases and medication, except for long-term, low-dose diuretic (hydrochlorothiazide) to control blood pressure which resulted in a reduction of blood pressure from 140/85 mmHg prior to medication to the 111/55 mmHg used in this study.

As described previously [48], [72], mice were laid supine after isoflurane inhalation anesthesia (2-3% for induction, 1.5% for maintenance) and secured on a surgical platform with a recirculating heating pad (TP-500 Heat Therapy Pump, Gaymar Industries Inc, Orchard Park, NY) to maintain body temperature at 37°C. Mean blood velocity and luminal diameters were then acquired via ultrasound (Vevo 2100 system, FUJIFILM VisualSonics) in the ascending thoracic aorta (ATA), infrarenal abdominal aorta (IAA), and a common carotid artery (CCA). Cardiac output (CO) was measured with standard B-mode transthoracic echocardiography and central aortic pressure was measured using a SPR-1000 Millar pressure catheter with a diameter of 1F. Hemodynamic measurements were performed on n =10 young adult and n = 10 old adult mice.

Aortic Biomechanics. The four-fiber family constitutive model described in Chapter 2 was used to quantify the biaxial material stiffness of the arterial wall, both human and murine. Since we could not (and should not) perform biaxial material testing on human subjects, the 8 model parameters and *in vivo* axial stretch (λ_z) were specified from literature data for three segments of the aorta: ATA, descending thoracic aorta (DTA), and IAA for different age groups [35], which corresponded to where PC-MRI measurements of area was collect. Material parameters for the young adult were based on data for 31–60 year old subjects, while those for the old adult were based on data for the 61+ year old age group. An iterative approach was then used to adjust the resulting pressure-diameter curves to match patient data on *in vivo* pressure and diameter. The unloaded diameter (and therefore the *in vivo* circumferential stretch (λ_θ)) was iteratively adjusted until the mean pressure (estimated as $\mathrm{P_{mean}} = (2\mathrm{P_{dias}} + \mathrm{P_{sys}})/3$ from cuff measurements) corresponded with the *in vivo* mean diameter (estimated as $\mathrm{D_{mean}} = (2\mathrm{D_{dias}} + \mathrm{D_{sys}})/3$ from PC-MRI images) on the pressure diameter curve [35]. Examples of adjusted pressure-diameter curves can be seen in Figure 3-2.



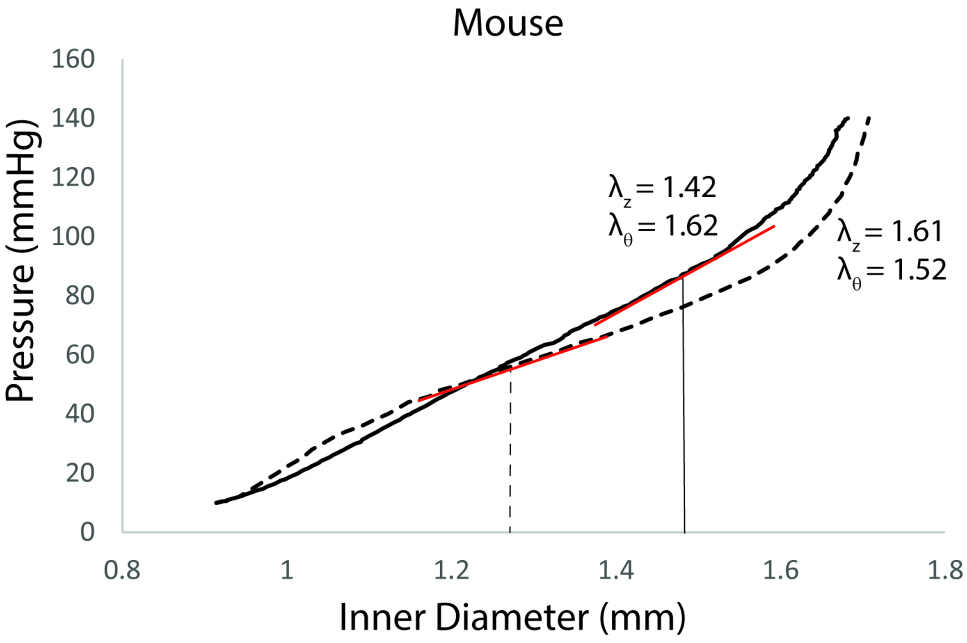


Figure 3-2. Pressure-diameter curves for the ascending thoracic aorta (ATA) of young (dashed line) and old (solid lines) adult subjects with corresponding values of in vivo *axial stretch (λ_z, from literature data) and circumferential stretch (λ_θ, iteratively calibrated). Linearized stiffness (red lines) were used in our FSI simulations. For the humans (top), curves are recreated from best-fit values of the 8 material properties from [35] with* in vivo *circumferential stretches adjusted until* in vivo *mean pressures corresponded with* in vivo *mean diameters. For the mice (bottom), the pressure-diameter curves were measured directly* in vitro*, with best-fit values of the material parameters determined via nonlinear regression of the biaxial data.*

For the mice, *ex vivo* mechanical testing was performed on four excised segments of the murine aorta (ATA, DTA, suprarenal abdominal aorta (SAA), and IAA) and a CCA with a computer-controlled custom biaxial testing device [87], as described previously [48]. After standard preconditioning, seven cyclic pressure diameter tests were performed: cyclic pressure diameter tests from 10 to 140 mmHg at three different fixed values of axial stretch (λ_z) (95%, 100%, 105% of the *in vivo* value) and cyclic axial extension tests at four fixed values of transmural pressure (10, 60, 100, and 140 mmHg) [26]. Best-fit values of the eight model parameters for the same four-fiber family constitutive model were determined for n = 5 young adult and n = 5 old adult mice [26].

3.2.2 Computational Modeling

FSI models were built with the open source computational hemodynamics platform CRIMSON [67]. As mentioned previously, data from imaging, hemodynamics, and wall mechanics were combined to create FSI models comprised of 3D anatomical models having spatially varying anisotropic wall mechanical properties and external tissue support, with inlet flow waveforms and 3-element Windkessel models on each outflow branch.

Anatomical Models and Finite Element Meshes. The CRIMSON GUI was used to create 3D models of the aorta and main branches. Centerline paths were determined for each vessel of interest and circular contours were added perpendicular to the centerline with discrete spacing to represent the vessel lumen. 3D volumes resulted by lofting between contours and applying a union function to blend the aorta and main branching vessels. For the humans, the 3D representation was based on MRA images. For the mice, the 3D representation was based on μCTA images. Furthermore, for the mice, 9 sets of intercostal arteries were added based on the location of the ribs.

Field-driven mesh adaptation techniques were used to create finite-element meshes refined on regions of high velocity gradients. For the humans, meshes had 1.4×10^6 tetrahedral elements and 2 x 10^5 nodes for the young adult and 2.6 x 10^6 tetrahedral elements and 5 x 10^5 nodes for the old adult. For the mice, meshes had 1.7 x 10^6 tetrahedral elements and 3 x 10^5 nodes for the young adult and 2.9 x 10^6 tetrahedral elements and 5 x 10^5 nodes for the old adult.

Boundary Conditions. For the humans, available data on pressure and cardiac output for each subject were used to inform computational models. For the mice, allometric scaling [48] was used to incorporate data from hemodynamics and wall mechanics from multiple mouse cohorts to the 3D FSI models. Briefly, allometric scaling took the form $\Upsilon = \Upsilon_0 \mathrm{M}^{\mathrm{b}}$ with Υ being the quantity of interest (e.g., CO, resistance R_{TOT}, and compliance C_{TOT}), Υ_0 the normalized quantity from experimental data, M the body mass, and b a scaling constant. Linear regression of log-log plots of the quantify of interest versus body mass was used to determine the coefficients [48].

Inflow Boundary Condition: For the humans, aortic inflow waveforms were generated from PC-MRI velocity and diameter data using the freely available Medviso software Segment, version 3.0 R8115 [88]. A semi-automated technique was used to segment the ATA lumen over one cardiac cycle. For the mice, population-averaged flow waveforms from a hemodynamics cohort were used, as described previously [48]. CO was allometrically scaled based on body mass for the old and young subjects, again as before [48], [89]. ATA flow waveforms and cardiac output can be seen in Figure 3-3 for all subjects.

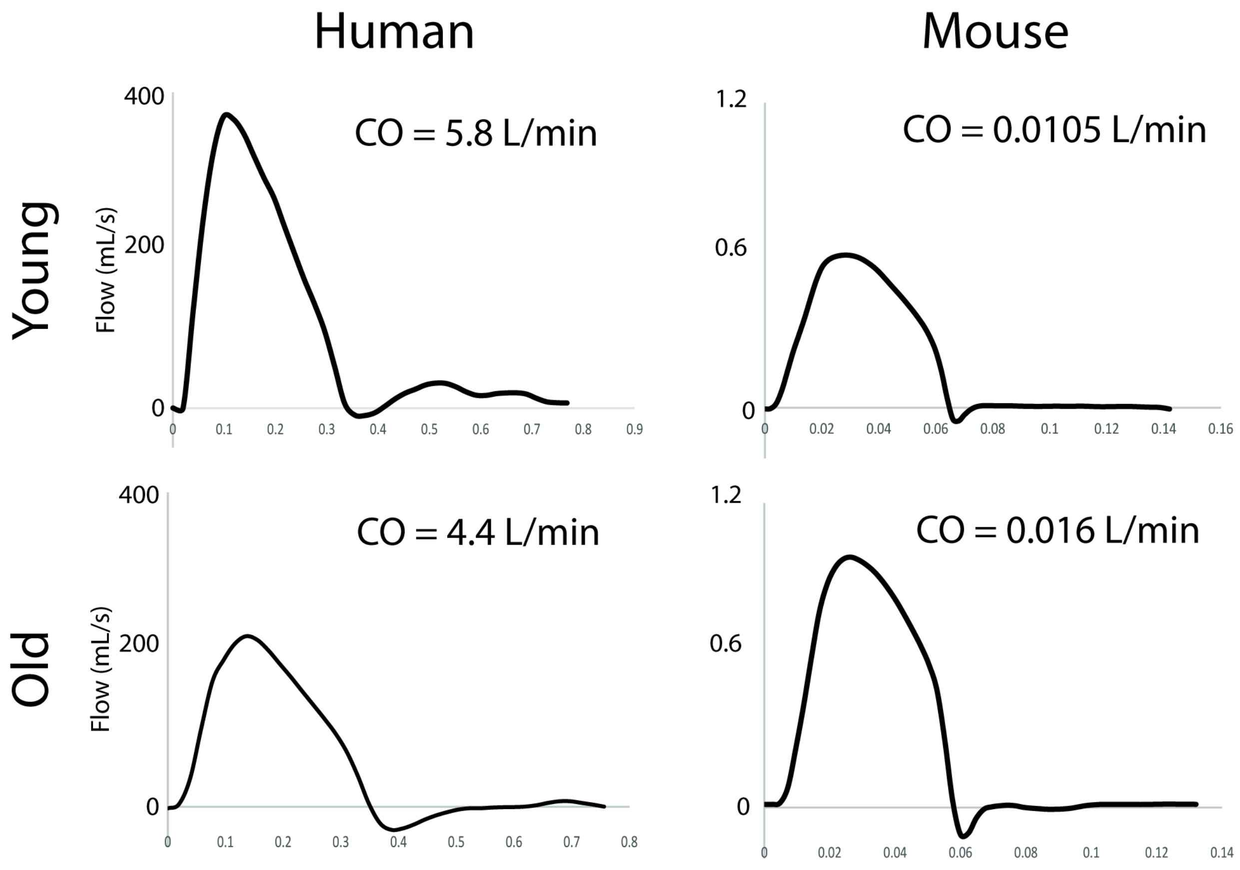


Figure 3-3. ATA inflow waveforms (mL/s) and associated values of CO (L/min).

Outflow Boundary Conditions: A three-element RCR Windkessel model was applied to the outlet of all branches. First, total arterial resistance (R_{TOT}) and total arterial compliance (C_{TOT}) were estimated. R_{TOT} was estimated from P_{mean}/CO for both species. For the humans, C_{TOT} was estimated from $(Q_{max} - Q_{min}/P_{systole} - P_{diastole})\Delta t$ [90]. For the mice, C_{TOT} was estimated from the slope of the diastolic decay curve of the experimental ATA pressure waveforms ($P_{diastole}(t) = P_0 \exp(-t/R_{TOT}C_{TOT})$) [76]. Furthermore, in the mice, R_{TOT} and C_{TOT} were estimated using population data from the hemodynamics and wall mechanics cohort, then allometrically scaled as described previously.

R_{TOT} and C_{TOT} can be separated into 3D (central) and peripheral portions. The 3D portion is set by the anatomy and stiffness of the central vasculature; the peripheral portion can be estimated iteratively. Once R_{TOT} and the distal portion of C_{TOT} are obtained, they must be distributed among the outflow branches of each computational model to ultimately specify proximal resistance (R^{i}_{prox}), compliance (C^{i}), and distal resistance (R^{i}_{dist}) for each outlet (i) as described previously [48], [75].

Wall Mechanics: The FSI simulations were based on a coupled momentum formulation [66]. The vessel walls were modeled as an incompressible, pseudoelastic membrane with a 5x5 stiffness matrix and wall thickness, h. The theory of small deformations superimposed on large was used to linearize the material stiffness around the mean pressure using *in vivo* axial and circumferential stretches [48], [71]. For both species, anisotropic stiffness parameters varied spatially for the aorta and main branches.

For the humans, thickness h and *in vivo* axial stretch were adopted from the literature: h was assumed to be 14% of the luminal radius for the young adult and 16% for the old adult [35]. Spatially varying parameters for the aorta were assigned for the ATA, DTA, and IAA, as described in 3.2.1. Linearization of stiffness occurred at the mean diameter and mean pressure point of the pressure diameter curve. Branching vessels were assigned the stiffness of the closest aortic segment; for example, the upper branches were assigned the stiffness of the ATA.

For the mice, h and *in vivo* axial stretch were determined from *in vitro* testing [26]. *In vivo* circumferential stretch was calculated from the unloaded diameter and pressurized diameter from μCT images. Linearization for the mice occurred at *in vivo* axial and circumferential stretches. For the ATA, *in vivo* axial stretch was determined from length in μCT images. Different values of stiffness and thickness were prescribed for six regions of the aorta, the ATA, proximal DTA (pDTA), distal DTA (dDTA), SAA, IAA and one CCA based on *in vitro* testing. The same values for the four-fiber family parameters were used for both sections of the DTA, but because of differences in diameter, linearization was performed at different *in vivo* circumferential stretch for the proximal and distal portions. Upper branches (both CCAs, left subclavian, and right innominate artery) were assigned the same stiffness and thickness as the tested CCA. Middle branches (mesenteric, celiac, and left and right renal arteries) were assigned the same stiffness matrix as the SAA and the thickness of the CCA. Iliac and tail arteries were assigned the stiffness and thickness of the IAA.

Perivascular support given by stiffness (k_s) and damping (c_s) coefficients were applied to all vessels to represent the pressure (P_{ext}) from tissues surrounding the vasculature [72], [73]. In the human, different k_s and c_s were applied to the ATA, DTA, and IAA portions of the aorta, as described previously [34]. Branches off the aorta were assigned the same values of perivascular support as the closest aortic segment. For the young adult human, k_s = 200 Pa/mm for the ATA, 100 Pa/mm for the DTA, and 10 Pa/mm for the IAA with c_s =10 Pa*s/mm for the entirety of the aorta. For the old adult human, k_s = 5200 Pa/mm for the ATA, 5050 Pa/mm for the DTA, and 10 Pa/mm for the IAA with c_s = 10 Pa*s/mm for the entirety of the aorta. A similar approach was used for the mice, as described previously for the young adult [48]. This resulted in applied k_s = 13500 Pa/mm for the ATA, 9000 Pa/mm for the pDTA, 16500 Pa/mm for the dDTA, 20000 Pa/mm for the SAA, 47000 Pa/mm for the IAA, and 50000 Pa/mm for the CCAs with c_s = 10 Pa*s/mm for the young adult mouse; global values of k_s = 40 Pa/mm and c_s = 30 Pa*s/mm were prescribed for the old adult mouse.

Pulse Wave Velocity Analysis: PWV was calculated from the ATA (near the aortic root) to the iliac artery (directly after the iliac bifurcation), which is different from the common carotid-femoral PWV (cfPWV) which fails to include the ATA, a region of particular change in aging [91]. PWV was calculated as the ratio of the centerline distance between the ATA and iliac artery and the pulse transient time (PTT). To calculate the PTT, the foot of the pressure waves at each region of interest was calculated based on the ‘intersecting tangent algorithm’ [92].

3.3 Results

3.3.1 Morphology

Anatomical models of the aorta and main branches of humans and mice can be seen in Figure 3-4(A). Body mass, height, and aortic length for all subjects along with age-related percent differences are also presented. Quantitative differences between young adult and old adult aortic inner diameter are shown in the bar plots for Figure 3-4(B), which show the percent diameter of each aortic region in comparison to the young ATA diameters. Humans experienced a more dramatic increase in aortic diameter than the mice, especially in the ATA though not in the IAA, noting that the aorta in the young human tapered gradually from the ATA to the IAA. This trend matches previously published work using human population data [34]. Both young and adult mouse geometries revealed the drastic reductions in IAA diameter in relation to the other regions.

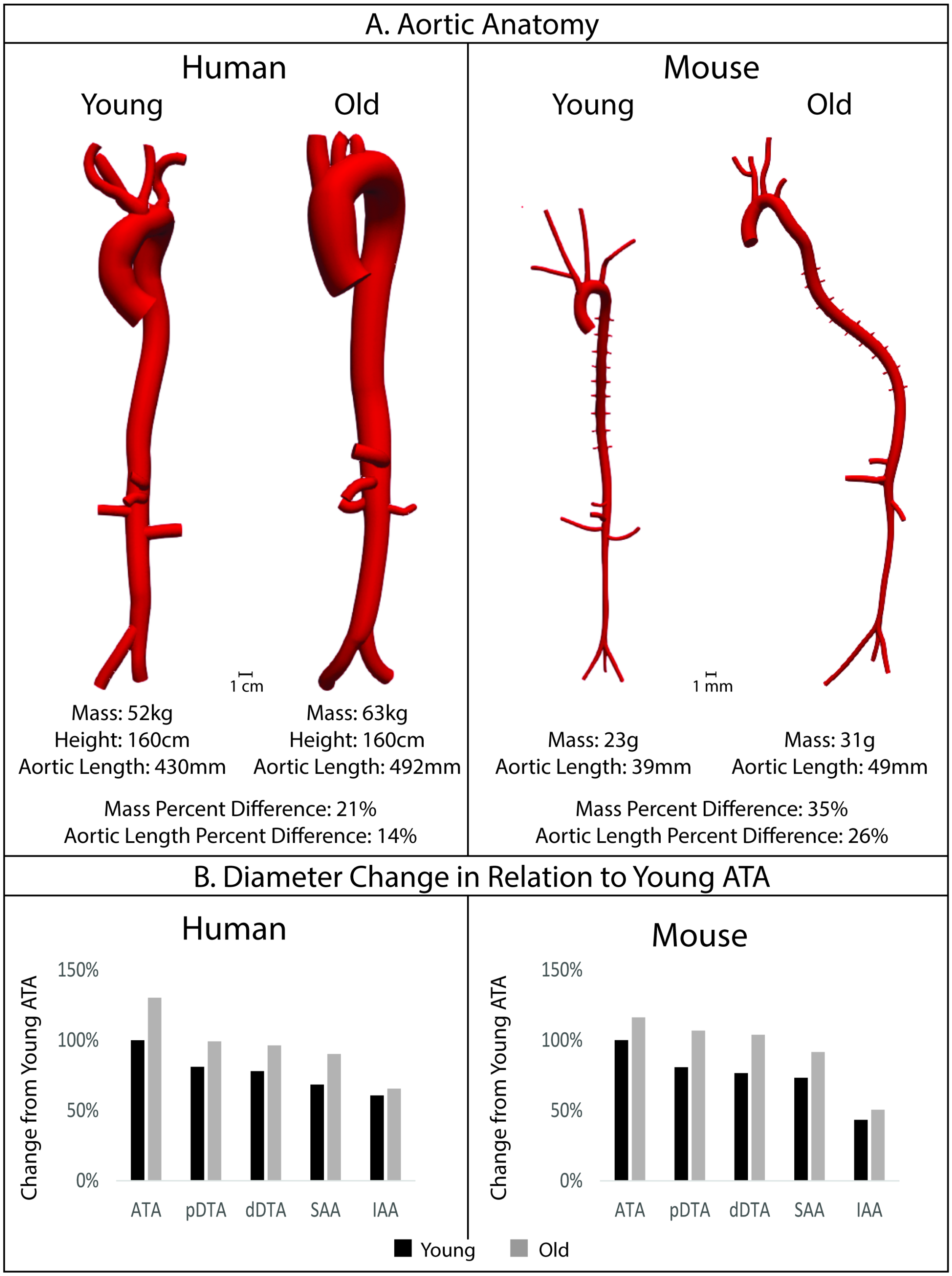


Figure 3-4. A: 3D models of the aorta and main branches for each subject along with body mass, height (for humans), and aortic length. Percent differences between young and old adults for each species is shown for mass and aortic length. Anatomical models are shown in the same scale between age groups, but not between species. B: Percent changes in inner diameter by region (proximal (pDTA) and distal (dDTA) descending thoracic aorta, suprarenal abdominal aorta (SAA), and infrarenal abdominal aorta (IAA)) of the aorta in comparison to the young ascending thoracic aorta (ATA) diameter for both species.

3.3.2 Material Properties

Figure 3-5 shows regional values of circumferential ($C_{\theta\theta\theta\theta}$) and axial ($C_{zzzz}$) material stiffness and wall thickness (h) prescribed based on available data (ATA, DTA, and IAA for humans based on linearized values from literature; ATA, pDTA, dDTA, SAA, and IAA for mice based on biaxial tissue testing). Humans and mice demonstrated differing patterns of aortic stiffness. Human subjects exhibited increased material stiffness down the aorta with peak values occurring in the IAA. In contrast, mice exhibited peak circumferential stiffness in the pDTA and then exhibited decreases distally. Nevertheless, both species experienced an increase in circumferential stiffness with age in all aortic regions with the exception of the human IAA. This increase in stiffness was larger in the mouse than the human with the largest increase (550%) occurring in the mouse pDTA. Humans had the greatest increase in circumferential and axial stiffness (87% and 606%, respectively) from young to old in the ATA. For the mouse, axial stiffness increased with age in all aortic regions except for the IAA. Wall thickness increased in the human subjects with age, while in the mouse it remained relatively constant, except for in the IAA.

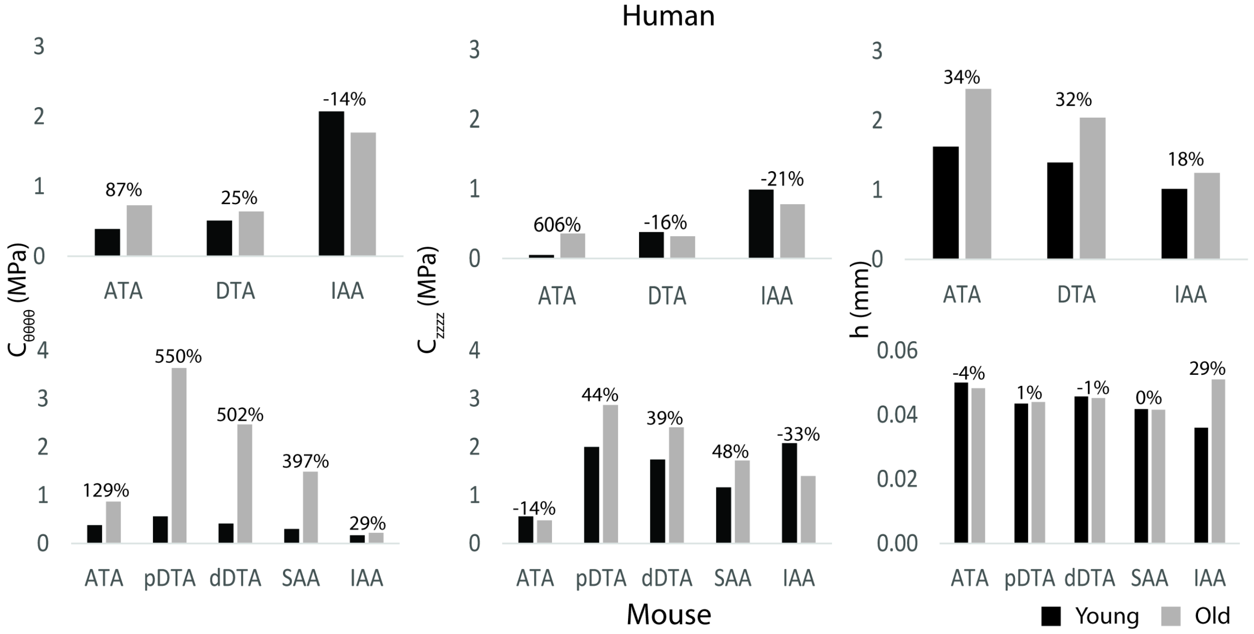


Figure 3-5. Regional circumferential ($C_{\theta\theta\theta\theta}$) and axial ($C_{zzzz}$) material stiffness and wall thickness h for young adult and old adult humans (top) and mice (bottom) for five locations along the aorta (ascending thoracic aorta (ATA), proximal (pDTA) and distal (dDTA) descending thoracic aorta, suprarenal abdominal aorta (SAA), and infrarenal abdominal aorta (IAA)). The percentages indicate changes with aging relative to the young value. Note that the ATA stiffened the most with aging in humans (cf. [91]) whereas the DTA stiffened the most with aging in mice.

3.3.3 Central and Peripheral Contributions of Resistance and Compliance

Figure 3-6 shows the breakdown of R_{TOT} and C_{TOT} between the central (3D) and peripheral components. R_{TOT} was of similar magnitude for mice and humans, but C_{TOT} was three orders of magnitude smaller for the mice. The young adult human had smaller total resistance and larger total compliance than the older. Conversely, mice experienced a decrease in resistance and an increase in compliance with age. Humans had most of their total compliance in the central vasculature, while mice, particularly the older mice, had a majority in the periphery. For both species, there was a decrease in central and increase in peripheral compliance with age.

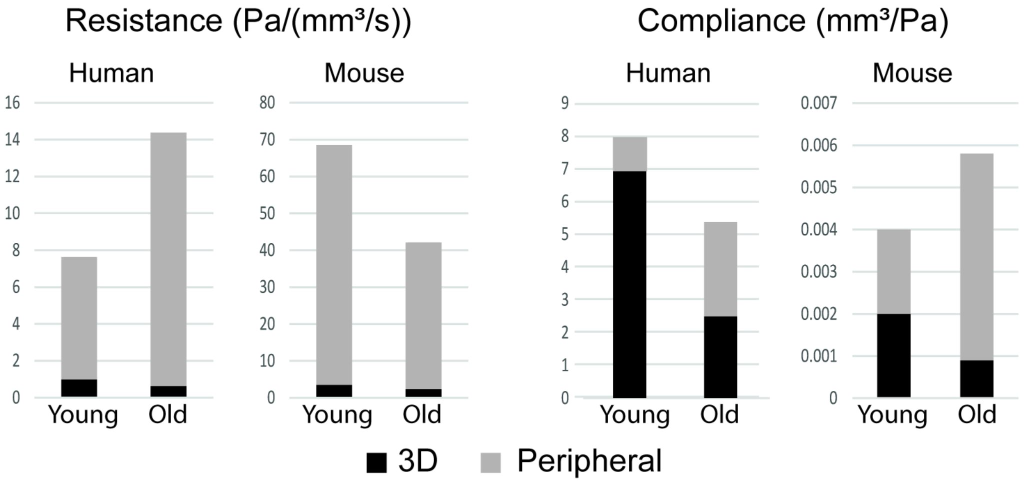


Figure 3-6. Total resistance R_{TOT} and compliance C_{TOT} broken into components of the central (3D) and peripheral vasculature.

3.3.4 Hemodynamics

Computational results for peak systolic blood velocity as well as regional (ATA, DTA, IAA) blood pressure and flow waveforms are shown in Figure 3-7 for all four models: young and old adult human, young and old adult mouse. The younger human had greater blood velocities than the older subject, consistent with the larger cardiac output (Figure 3-3) and smaller aortic dimensions (Figure 3-4). Conversely, the older mouse had greater blood velocities and higher aortic flows than the younger. Both older subjects show reverse flow in diastole, which is absent in the younger subjects. Human CO decreased 32% from the young to the old subjects, while mouse CO increased 52% with age.

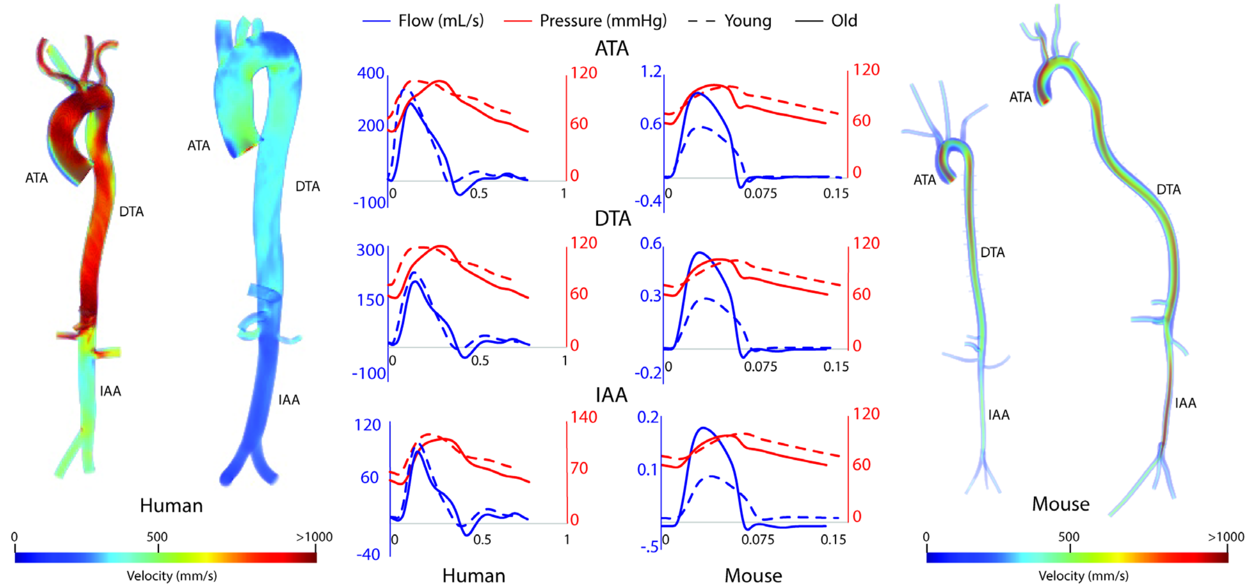


Figure 3-7. Volume rendering of blood velocity at peak systole for the humans (left) and mice (right), with pressure and flow waveforms at three sites along the aorta (ascending thoracic aorta (ATA), descending thoracic aorta (DTA), and infrarenal abdominal aorta (IAA)) (center).

Hemodynamic results were validated against experimental data. For the humans, averaged blood pressure cuff measurements were compared with the simulated (not imposed) left subclavian artery pressure (Figure 3-8), resulting in discrepancies less than 5% for diastolic and systolic pressures for both young and old adult humans. For the mice, simulated ATA pressure waveforms were compared with measured Millar ATA pressure waveforms in terms of pulse pressure, mean pressure, and slope of the diastolic decay (Figure 3-8). Further, simulated IAA and CAA mean flows showed good agreement with their experimental counterparts.

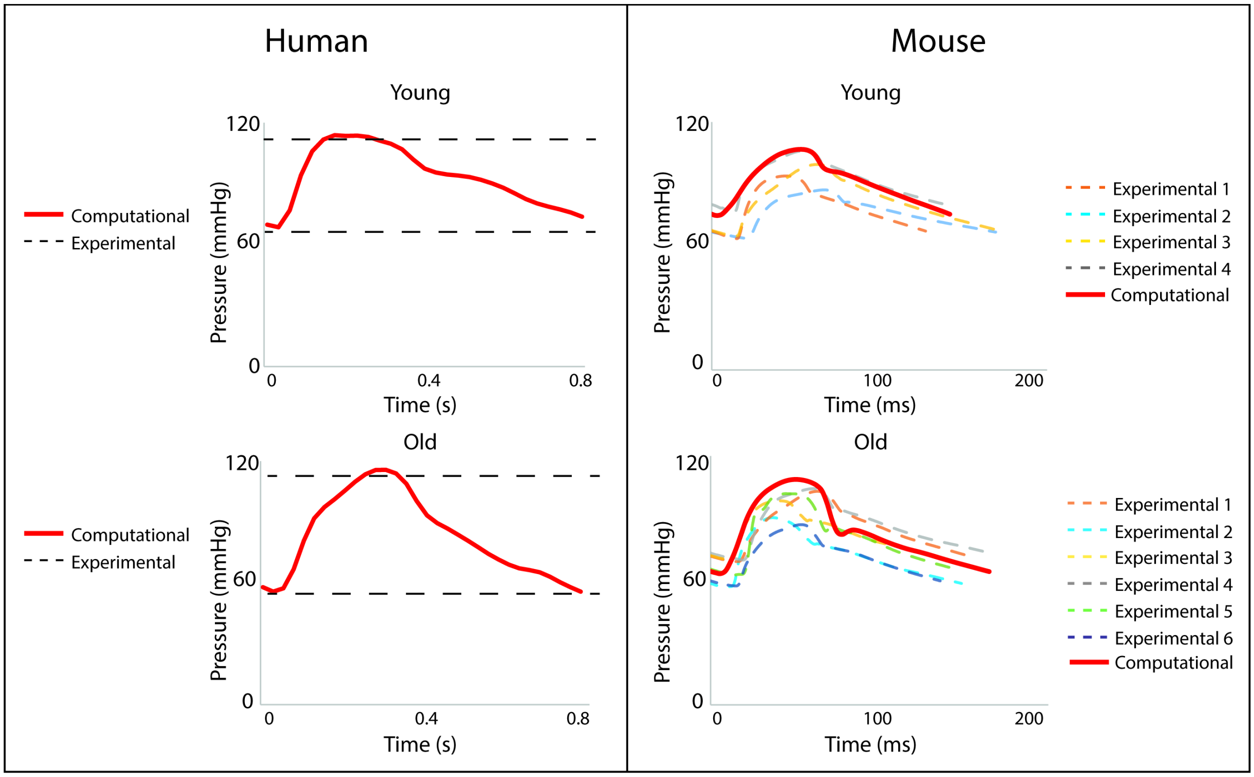


Figure 3-8. Validation of hemodynamic results using pressure data. For humans, the simulated left subclavian artery pressure (solid red line) compared well with systolic and diastolic pressure cuff values (dashed black lines). For the mice, simulated ascending thoracic aortic (ATA) flow waveforms (solid red lines) compared well with experimental data from multiple subjects (dashed lines).

Figure 3-9 shows the calculated mean arterial pressure for five aortic locations (ATA, pDTA, dDTA, SAA, IAA) and iliac artery. Mean pressure was similar between species and the pressure gradient down the aorta was small for all subjects. Figure 3-10 shows pressure waveforms at the same six locations. Numerical values for pulse pressure are indicated at the ATA and iliac artery. The overall spatial trends of the pressure waveforms differ significantly between species. Humans show an amplification of the pulse pressure down the aorta (31% increase for the young, 9% increase for the old, see table in Figure 3-10), consistent with the increase in material stiffness reported in Figure 4. On the other hand, mice show an attenuation of pulse pressure down the aorta (-19% for the young, -36% for the old), consistent with the reported decrease in material stiffness in the descending thoracic and infrarenal aorta (Figure 3-5). Conversely, both species show similar changes in ATA pulse pressure with aging: 33% increase for the old human and 41% increase for the old mouse. These increases in pulse pressure are driven by the similar stiffening of the ATA with age (87% increase in circumferential stiffness for the human, 129% increase for the mice, see Figure 3-5). Lastly, ATA-to-iliac PWV and spatially weighted averages of structural aortic stiffness can be seen in Figure 3-11. The humans had higher values of PWV than the mice. Yet, the mice showed a larger increase in PWV with age than did humans, consistent with the larger increase in structural stiffness.

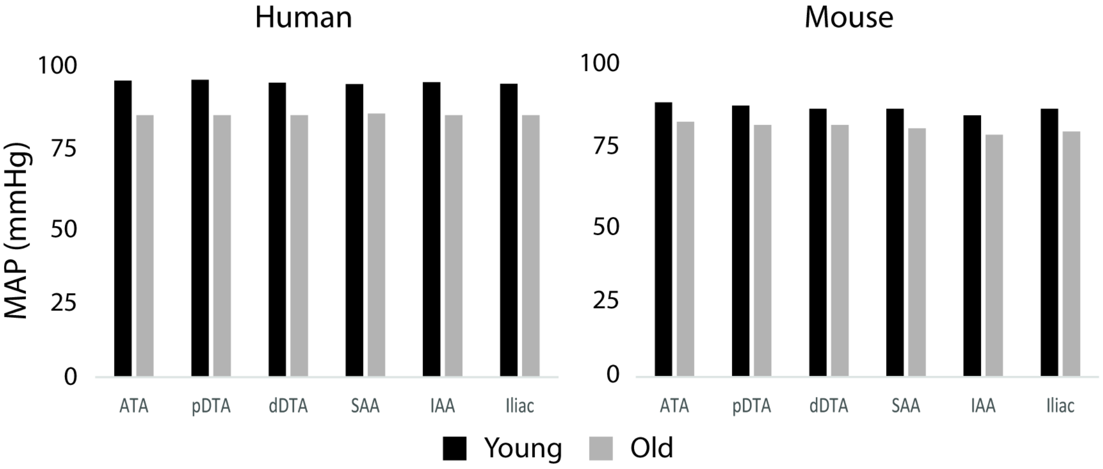
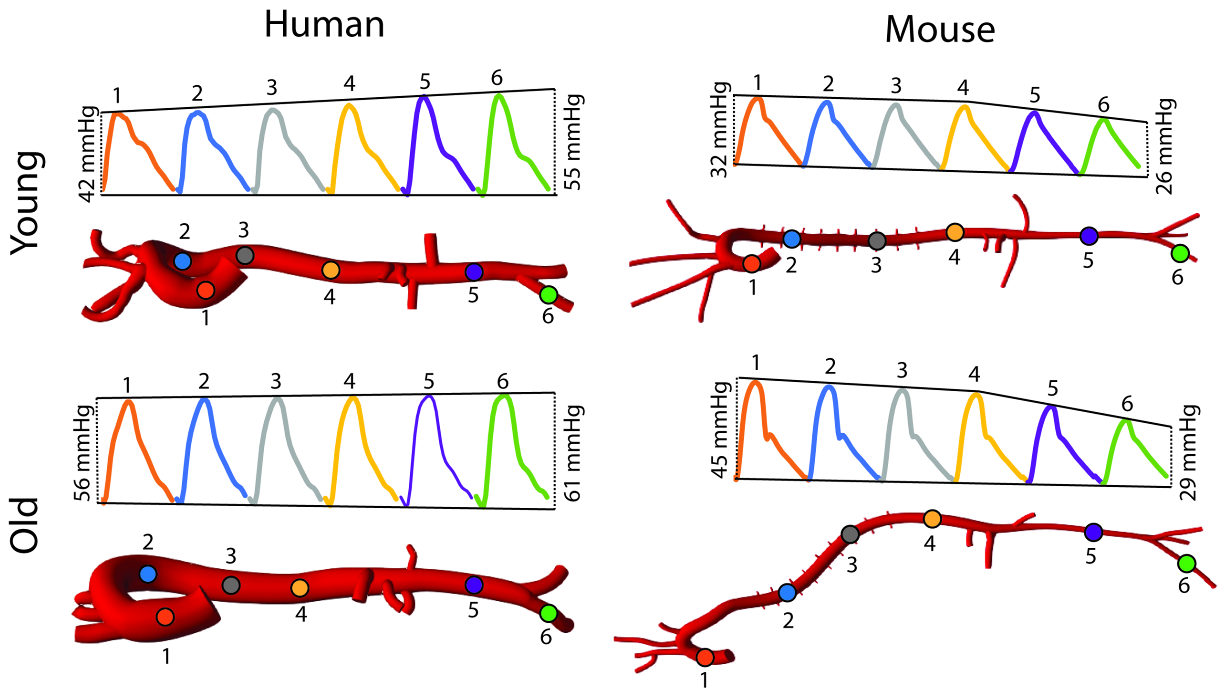


Figure 3-9. Computed values of mean arterial pressure (MAP) for five aortic locations (ascending thoracic aorta (ATA), proximal (pDTA) and distal (dDTA) descending thoracic aorta, suprarenal abdominal aorta (SAA), and infrarenal abdominal aorta (IAA)) and an iliac artery for all four primary study groups, all showing modest changes proximal to distal.



	Human			Mouse		
	Young	Old	% Difference with Age	Young	Old	% Difference with Age
ATA Pulse Pressure (mmHg)	42	56	+33	32	45	+41
Iliac Pulse Pressure (mmHg)	55	61	+11	26	29	+12
Pulse Amplification (%)	+31	+9		-19	-36	

Figure 3-10. Pulse pressure amplification manifests in the human aorta, but not the mouse.

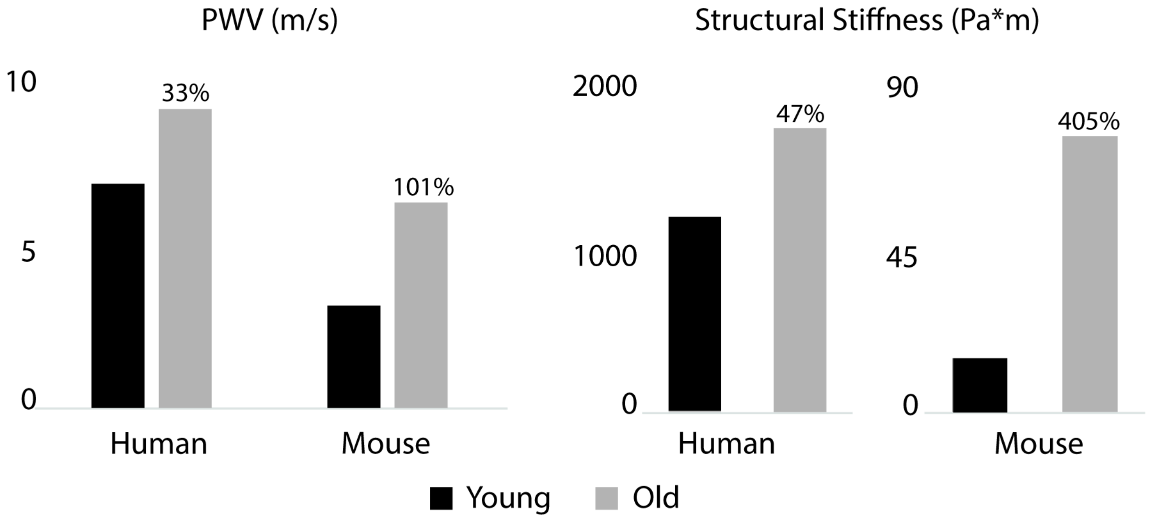


Figure 3-11. Aorto-iliac PWV (left) and spatially weighted averages of structural aortic stiffness (right) for the humans and mice. Percent difference from young to old are indicated for both species. Baseline values for the young subjects were 6.9 m/s for human and 3.2 m/s for mouse PWV and 1195.7 and 15.3 for human and mouse structural stiffness.

3.4 Discussion

Choice of murine model is an important factor to consider for ensuring applicability of results to the human disease condition. In this study, we used technical action research to improve the generalizability of results from murine models for cardiovascular aging research. FSI modeling of the cardiovascular system was used to compare the hemodynamic and structural effects of natural aortic aging between humans and mice. The differences and similarities discussed below can inform future murine studies of cardiovascular aging. To our knowledge, this study is the first comparison of the differences in hemodynamics, wall properties, and vascular anatomy as a function of age between mice and humans. In the following paragraphs, the results for our representative subjects are compared to population-based data from literature, the similarities and differences between humans and mice in terms of aging are analyzed in depth, and the limitations to the current study are described. A discussion of the inferences from this analysis to future mouse studies can be found in Chapter 5.

The representative healthy human subjects in this study showed a decrease in CO with aging, which is consistent with trends found in the literature [93]. Consistent reporting of changes in CO and other hemodynamic parameters with age in mice has been challenging due to variations in strains and experimental procedures [80]. Here, mice showed an increase in CO with age which can be explained by the increase in body size [85]. Furthermore, both older subjects had reverse flow throughout the aorta, which is absent in the younger subjects. This is of particular importance in the IAA, as reverse flow negatively impacts renal function [94].

In this study, older subjects showed larger values of aortic length and diameter for both species. In the human, this is consistent with trends found in the literature [95], [96]. For the representative subjects used herein, aortic length increased more in the mice than in the humans because of the increased aortic tortuosity and body size in the older mouse. Aortic tortuosity often increases with age in humans as well [34], [51] although this was not seen in the old adult human subject in this study. There was a trend toward a decreasing aortic inner diameter along the length of the aorta in both species, which is consistent with results reported in previous studies [34], [35].

Humans and mice showed marked differences in both spatial patterns of aortic stiffness and changes in stiffness with aging. Mainly, we see an increase in stiffness down the aorta in humans, which is consistent with trends found in literature [25], [35]. In contrast, in mice the circumferential stiffness increases from the ATA to the pDTA, then decreases distally, which is consistent with previous murine studies [48], [97]. With age, both species exhibit an increase in aortic stiffness. In the humans, the largest increase in stiffness with age occurs in the ATA. In the mice, the largest stiffening happens in the DTA and SAA. These age-related changes in stiffness of the two species are most likely determined by different mechanisms: mechanical fatigue-induced loss of elastic fiber integrity over many decades in the human [31], [79], [83], and accumulation of proteoglycans and remodeled collagen in the mouse [84], [85].

The aforementioned differences in aortic stiffness between species and age groups also resulted in marked hemodynamic differences. Consistent with previously reported findings [98], [99], humans present a pulse pressure amplification down the aorta while mice have pulse pressure attenuation down the aorta. However, both species showed an increase in ATA pulse pressure with age, which suggests similar hemodynamic responses to aging. In the humans, ATA and iliac pulse pressures became more similar with age, also consistent with prior findings [98], [99], whereas pulse pressures along the aorta became more different in the mouse with age.

Both species showed an increase in ATA to iliac PWV with age. Despite not being commonly used clinically due to difficulty in measurement, aortoiliac PWV has advantages over cfPWV, mainly that it includes the ATA, an area known for stiffening with age [25], [91]. For the humans, PWV values were consistent with mean healthy population data 50 for both age groups: 6.2 ± 1.5 m/s (compared to our 6.9 m/s) for 30-year-olds, and 10.9 ± 5.4 m/s (compared to our 9.23 m/s) for subjects > 70-year-old subjects. While ATA to iliac PWV was directly calculated from our computational models, [100] measured cfPWV and scaled it by 0.8 to represent the ATA to iliac values.

Humans and mice also exhibited different trends in terms of total arterial resistance and compliance. Figure 5 shows that most of the vascular compliance is in the periphery in mice, as opposed to in the central vasculature in humans [101]. The younger human has lower total resistance and larger total compliance than the older, which contradicts the trend seen in the mouse of a decrease in resistance and an increase in compliance with age. These findings in mice are consistent with previous computational FSI studies of hemodynamics in fibulin-5 deficient mice

[48]. Despite an overall decrease in vascular resistance and increase in vascular compliance, there is an increase in resistance and decrease in compliance in the central vasculature, which is consistent with our age-related changes in vascular diameter and stiffness.

Despite the importance and advantages of direct comparisons across species and age using consistent methods, limitations herein include the use of subject-specific morphological and hemodynamic data for the humans (noting expectation of considerable differences across subject cohorts) and population-based data for the mice. In this work, we do not present any population-based statistics. Statistical significance would be important to address for different patient cohorts or for different mouse groups, but with greater numbers of subjects. The key focus here is not on group-to-group differences, but rather on similarities and differences between representative subjects of the species, human and murine in particular. Furthermore, in both species, nonlinear material properties were population-based. For the humans, the parameters of the 4-fiber family model were the only non-subject specific data used to build the computational models, although linearization was informed by subject-specific deformations. While trends in stiffness and PWV are consistent between our subjects and literature, the impact of the population-based data should be further explored. For example, the method to linearize stiffness with our subject-specific deformations should be perform with multiple age-based parameters included in [35] to determine the effects on trends in stiffness and resulting hemodynamics. All results were for females, and cardiovascular aging likely differs for males. Additionally, the older human subject has experienced menopause and the related decrease in circulating sex hormones, which is known to impact cardiovascular health [102]. In contrast, the naturally aged mouse model did not experience menopause. To better incorporate the effects of menopause, ovariectomized aged female mice could be used in future studies [103]. Similar studies comparing young and old males and contrasting aged males and females would be interesting as well.

Because we used population-based data for a particular mouse colony, our results hold for the *C57BL/6 x 129/SvEv* strain, with expected differences for pure *C57BL/6* and *129/SvEv* mice (cf. [104]). Previous work has characterized regional aortic biomechanics in multiple mouse models, though without associated FSI analyses of the central hemodynamics [26], [97], [105], [106]. It was beyond the present scope to consider effects of specific genetic mutations or modifiers in either the mice or the humans. Moreover, because we used subject-based data for the human, these results are not indicative of the entire population, although the subjects were selected such that changes in cardiac output, aortic dimensions, and aortic stiffness with age were consistent with reported population data. The only notable difference is the lack of increased tortuosity in the older human subject aorta. Furthermore, the *in vivo* data were collected under resting conditions in the human and under anesthesia in the mice. Finally, because of the ability to use invasive procedures in murine subjects, there are intrinsic differences in the measurement techniques between the species. To mitigate the effects of this, efforts were made to ensure the consistency of data. Particularly, despite not being able to perform biaxial tissue testing in humans, the four-fiber family model was used based on literature values and adapted to simulated biaxial test data [35]. Computational techniques were consistent between the species.

Chapter 4 Generalizability of Results for Studying Hypertension: Impact of Isoflurane Anesthesia with Angiotensin-II Infused Mice.

4.1 Introduction

Anesthesia is necessary for murine measurements that require the animal to be still or causes discomfort, which includes hemodynamic assessments using echocardiography, ultrasound, microCT, and catheter-based assessment of blood pressure. For cardiovascular studies aiming to capture *in vivo* hemodynamics, the effects of anesthesia need to be considered in the analysis of results, as hemodynamics will differ from the typical awake state. While the exact mechanisms of action for many anesthetics are unknown, many involve direct depression of cardiac output or reduction of systemic vascular resistance (SVR) [107], [108]. No matter the specific mechanisms of action, vascular relaxation is expected with anesthesia, which reduces mean arterial pressure (MAP) leading to a decrease in arterial luminal diameter and passive vascular stiffness, based on the vascular stress-strain relationship, and an increase in wall thickness, based on the assumed incompressibility of the vasculature wall. Inhalant anesthetics, including isoflurane and halothane, are commonly used for murine studies, as it is easy to monitor and adjust anesthetic depth and recovery is quick [64]. Isoflurane is typically the preferred inhalant anesthetic in murine studies, as it has been reported to maintain physiologic stability, in terms of heart rate, blood pH, and arterial pressures of O_2 and CO_2 [109]. While the exact mechanisms of action of isoflurane are unknown [108], there is known reduction in contractility of cardiac and smooth muscle cells, which depresses cardiac function and decreases SVR to result in a reduction of MAP [110].

For murine studies comparing *in vivo* hemodynamics between controls and disease mouse models, the effect of anesthetic can safely be ignored if the reduction in hemodynamic parameters is similar between cohorts. However, previous studies have reported complex interactions between isoflurane and angiotensin-II (AngII), a common mouse model for studying hypertension. AngII is a critical molecule of the renin-angiotensin-aldosterone-system (RAAS), which maintains homeostatic blood perfusion to the kidneys [111]. Dysfunction or dysregulation of the RAAS system increasing the amount of circling AngII is common in many clinical presentations of chronic systemic hypertension; therefore, there are multiple therapies that interact with this system, including angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) [112]. AngII-infused mouse models have been used to study the effects of systemic hypertension as well as to promote atherosclerosis, aortic aneurysms, and dissections [97], [113]. AngII-infusion effects many bodily systems, including the brain, heart, vasculature, kidneys, gut, musculature, etc. [114]. In the vasculature, it is known that AngII induces vasoconstriction, which increases SVR leading to higher systemic blood pressure and increasing stiffness through the feedback loop discussed in Chapter 2. In addition, AngII directly causes aortic stiffening through inflammation in the vasculature wall [97], [115], which has been shown to vary based on aortic region [97].

The effects of the reported regional variations in aortic stiffness on local hemodynamics are not well known. Further, because of the clinical relevance of and widespread use of AngII-infused mouse models, it's important to be able to classify *in vivo* hemodynamics, which may lead to insights into the progression of advanced systemic hypertension and secondary cardiovascular diseases. It is therefore important to understand the reported interactions between isoflurane and AngII-infusion. Previous studies have reported that isoflurane dampens the hemodynamic and vasoconstrictive responses to acute infusion of AngII [116]–[118]. The cause of this has been attributed to isoflurane inhibiting Ca^{+2} handling in aortic smooth muscle cells as a result of AngII, i.e., decreasing the amount of Ca^{+2} to be released from the sarcoplasmic reticulum or cross the cell membrane, which reduces the ability of vascular smooth muscle cells to contract [117], [119]. Despite evidence for this strong effect of isoflurane on AngII-mediated changes in hemodynamics, little is known about the acute effects of isoflurane on the measurement of hemodynamics in mice chronically infused with AngII.

In this study, we use an action research framework to improve the generalizability of results from murine experimental studies that use AngII-infusion and isoflurane anesthesia, which can be seen in Figure 4-1. The problem identified is the potential differential effects of isoflurane anesthesia on chronically AngII-infused mice. An intervention was developed to analyze this effect, which included a workflow to estimate the awake parameters from FSI models built with anesthetized data. This workflow included iterative adjustment of peripheral resistance and compliance with aortic stiffness and diameter influenced by the direct and indirect effects of isoflurane based on awake tail cuff pressure measurements. Awake and anesthetized hemodynamics and vascular structure were compared for control and AngII-infused mice, leading to recommendations to improve future murine experiments and insights about the effects of isoflurane, AngII-infusion, and mouse-to-mouse variations in hypertension phenotype.

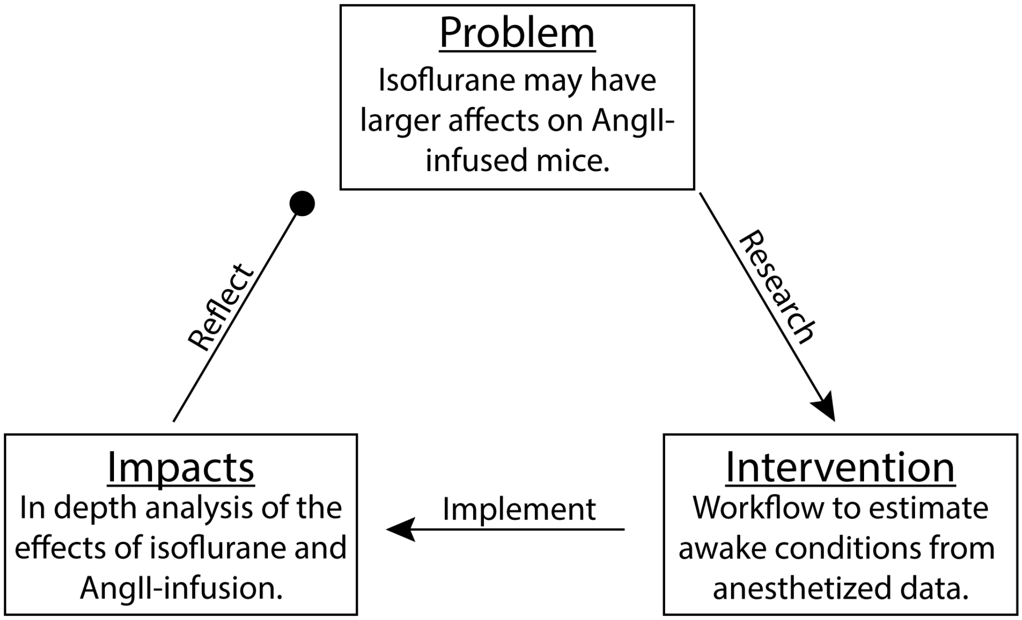


Figure 4-1. Action research cycle for improving the generalizability of results from murine studies with AngII-infused mice and isoflurane anesthesia. In this study, we stop after the reflection stage with recommendations for improving experiments and continuing the cycle.

4.2 Methods

All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Yale University. While under deep anesthesia, mini-osmotic pumps were implanted subcutaneously in three experimental mice to deliver AngII continuously at 1000 ng/kg/min for 14 days. Three non-implanted, non-infused mice served as controls. All mice were male on a *C57BL/6J* background. Both groups were euthanized at 14 weeks of age using Beuthanasia-D and death was ensured by exsanguination upon removal of the aorta for biomechanical testing.

4.2.1 Experimental

To promote rigor and reproducibility, the *in vivo* and *ex vivo* experimental procedures followed prior protocols [32], [48], [72] with the exception that all experimental procedures were performed on each mouse used in the study, hence allowing direct matching of regional mechanical properties and *in vivo* hemodynamics. Briefly, tail-cuff measurements (Kent Scientific, CT) of peripheral blood pressure were taken prior to and after 14 days of AngII infusion under awake conditions. To minimize variations in these measurements, mice were preconditioned to the system for multiple days prior to each actual measurement. Next, the mice were anesthetized with isoflurane (2.0-4.0 % for induction, 0.5-1.0 % for maintenance) and imaged using ultrasound (Vevo 2100, Fujifilm VisualSonics) to collect information on local blood flow (including cardiac output) and arterial diameter at key sites including the ascending aorta, infrarenal abdominal aorta, and left common carotid artery. The mice were then allowed to recover. The next day, the mice were re-anesthetized and a bolus intravenous (tail vein) injection of a nanoemulsion formulation (Fenestra VC, MediLumine Inc, Montreal) at 0.2 ml/20 g was used to obtain microCT images (eXplore CT120, GE Healthcare) to visualize the entire central vasculature, from carotid to the iliac arteries. Finally, while still under anesthesia, a SPR-1000 Millar pressure catheter was advanced from a right carotid cut-down to mid-ascending aorta to measure central blood pressure. Once complete, the mice were euthanized and the entire aorta plus the common carotid arteries were excised.

The excised vasculature was divided into five segments: left common carotid artery (LCCA), ascending (ATA) and descending (DTA) thoracic aorta, and suprarenal (SAA) and infrarenal (IAA) abdominal aorta. Each segment was separately cleaned of excess perivascular tissue, then cannulated on custom micropipettes and placed within a custom biaxial testing device [87] within a Hank's buffered physiological solution at room temperature to ensure passive mechanical behaviors. Following a standard preconditioning protocol, each segment was subjected to 7 testing protocols: 3 cyclic pressure-diameter tests while held at, or ±5% of, the *in vivo* value of axial stretch plus 4 cyclic axial force-length tests while held fixed at 10, 60, 100, or 140 mmHg. The pressure, outer diameter, axial force, and axial extension were collected on-line, yielding over

2800 data points per sample. Data from the unloading portion of the last cycle of each protocol were then used to find best-fit values of 8 material parameters in an independently validated "four-fiber family" constitutive relation, as described in Chapter 2. Please see our prior papers for further details, including characterizations across regions of the central vasculature [32], [97].

4.2.2 Anesthetized Computations

Multi-domain fluid-solid-interaction (FSI) models were created using the open-source finite element modeling platform CRIMSON [67]. Importantly, these models were informed using the microCT, ultrasound, and invasive catheter data, and necessarily defined the anesthetized state. These *in vivo* data on vascular anatomy, blood flow, and blood pressure were augmented with the *ex vivo* measurements of regional wall dimensions and material properties to define a spatially varying, deformable, three-dimensional (3D) computational domain, which was augmented further using estimated values of external tissue support. A measured pulsatile inflow waveform was prescribed at the ascending aorta, and 3-element Windkessel (RCR) models were applied at all key branch outlets. Parameters of the Windkessel models were tuned iteratively until computational values for the ATA pressure, IAA flow, and CCA flow were within 10% of experimental values. Please see our prior studies for further details on the associated methods [48], [120] as well as Chapter 3, but below find some additional details of importance below.

Anatomical Models. Six mouse-specific models (3 controls, 3 hypertensive) were defined by 3D representations of the aorta and main branches, which were segmented manually from the microCT images using a CRIMSON interface. Path-lines were drawn along the center of the vessels of interest; 2D contours were added perpendicular to the path-lines to represent the vessel lumen; the contours were lofted to create 3D volumes for each vessel and vessels were blended to create a 3D model of the central vasculature. Nine pairs of intercostal arteries were added based on the location of the ribs. Tetrahedral finite element meshes were generated for each of the six 3D anatomical models, each typically consisting of over 1.6 million elements. Field-driven adaptation was used to create meshes based on regions of high velocity gradients.

Wall Mechanics. As mentioned previously, a coupled momentum formulation was used for the FSI simulations [66]. The vascular walls were modeled as incompressible and pseudoelastic, with regionally specific anisotropic properties defined by a 5 x 5 stiffness matrix and local wall thickness, h. Regional values of stiffness were determined via a “small on large” linearization of the nonlinear material properties [71] determined for each of separate regions for each of the six samples that were tested *ex vivo*. The *in vivo* values of axial stretch and wall thickness were also determined from the biaxial tissue testing except for the ATA. The *in vivo* axial stretch of the ATA under anesthetized, rest, and normal ambulatory conditions is expected to be less than the experimentally determined energetically optimal value, which would be expected to hold under extreme “fight or flight” conditions. Thus, the ATA stretch was estimated based on microCT and ultrasound values [48]. The value of the *in vivo* circumferential stretch was determined from the unloaded diameter measured *ex vivo* and the pressurized, anesthetized diameter from the microCT images. Stiffness and wall thickness were prescribed at six locations of the aorta: ATA, proximal DTA (pDTA), distal DTA (dDTA), SAA, and IAA, and both CCAs based on results from biaxial tissue testing. Branches were assumed to have values of stiffness based on the closest region of the aorta, as found to be useful previously [120]. Effects of perivascular support were modeled using stiffness (k_s) and damping (c_s) coefficients [48], [72], [73]. Values of these coefficients were tuned iteratively, along with parameters for the Windkessel models (see below), to match the measured ATA pressure.

Boundary Conditions. Inlet flow waveforms were prescribed based on ultrasound-measured velocity and diameter at the ATA. Cardiac output (CO) was calculated from these waveforms; if it differed more than 10% from the CO measured from echocardiography, it was scaled to match the experimental value. Furthermore, based on prior findings [89], CO was scaled by 7% to account for the hemodynamic impact of the Millar catheter during pressure measurements. Because previous studies have reported limited effect of isoflurane anesthesia on CO [107], [121]–[123]. inflow waveforms were kept consistent with the anesthetized measurements for awake simulations.

A 3-element RCR Windkessel model, consisting of distal and proximal values of resistances (R_d and R_p, respectively) and compliance (C), was applied at the outlet of each of the aortic branches to represent the vasculature truncated from the 3D model. Values of the Windkessel parameters were first estimated based on experimental data, as described previously [48], [90], [120]. Briefly, total resistance (R_{TOT}) was estimated as P_{mean}/CO while total compliance (C_{TOT}) was estimated based on the slope of the diastolic decay curve of the ATA pressure waveform, using $P_{dias}(t) = P_0 \exp\left(-\frac{t}{R_{TOT}C_{TOT}}\right)$ [76]. R_{TOT} and C_{TOT} were then separated into a 3D and peripheral component and were distributed to outlet branches as described previously [90]. The values of the Windkessel parameters were then adjusted iteratively until computational simulations matched the measured values of ATA pressure and mean flow in the IAA and CCA, as available. All mice had sufficient data to match mean flow in the IAA, while two mice (AngII - A1 and control - C3) did not have sufficient data to estimate mean flow in the CCA.

4.2.3 Awake Computations

After successful calibration of the FSI models to match the anesthetized experimental data, the six models were adjusted to represent awake conditions, first, to determine the effects of isoflurane anesthesia on the hemodynamics in both the control and AngII-infused mice and, second, to evaluate effects of the AngII-induced vascular remodeling on the hemodynamics in the more relevant, awake, state. To achieve this, an iterative workflow was developed to adjust the anesthetized model parameters (R_{TOT}, C_{TOT}, linearized stiffness $\mathbb{C}$, wall thickness h, and luminal diameter d) based on the mouse-specific awake tail-cuff pressure data. The steps of the workflow (Figure 4-2) include:

1. update R_{TOT} and C_{TOT} based on the difference between the computationally simulated tail waveform and the experimental data. Run simulation,
2. update d and $\mathbb{C}$ based on the new simulated pressure and the pressure-diameter curve from *ex vivo* testing. Run simulation, and
3. calculate the errors for systolic (ε_{sys}) and diastolic (ε_{dias}) values of the tail pressure and changes in R_{TOT} (Δw_R) and C_{TOT} (Δw_C). If pressure errors and changes in Windkessel parameters are greater than 5%, the repeat (1-3).

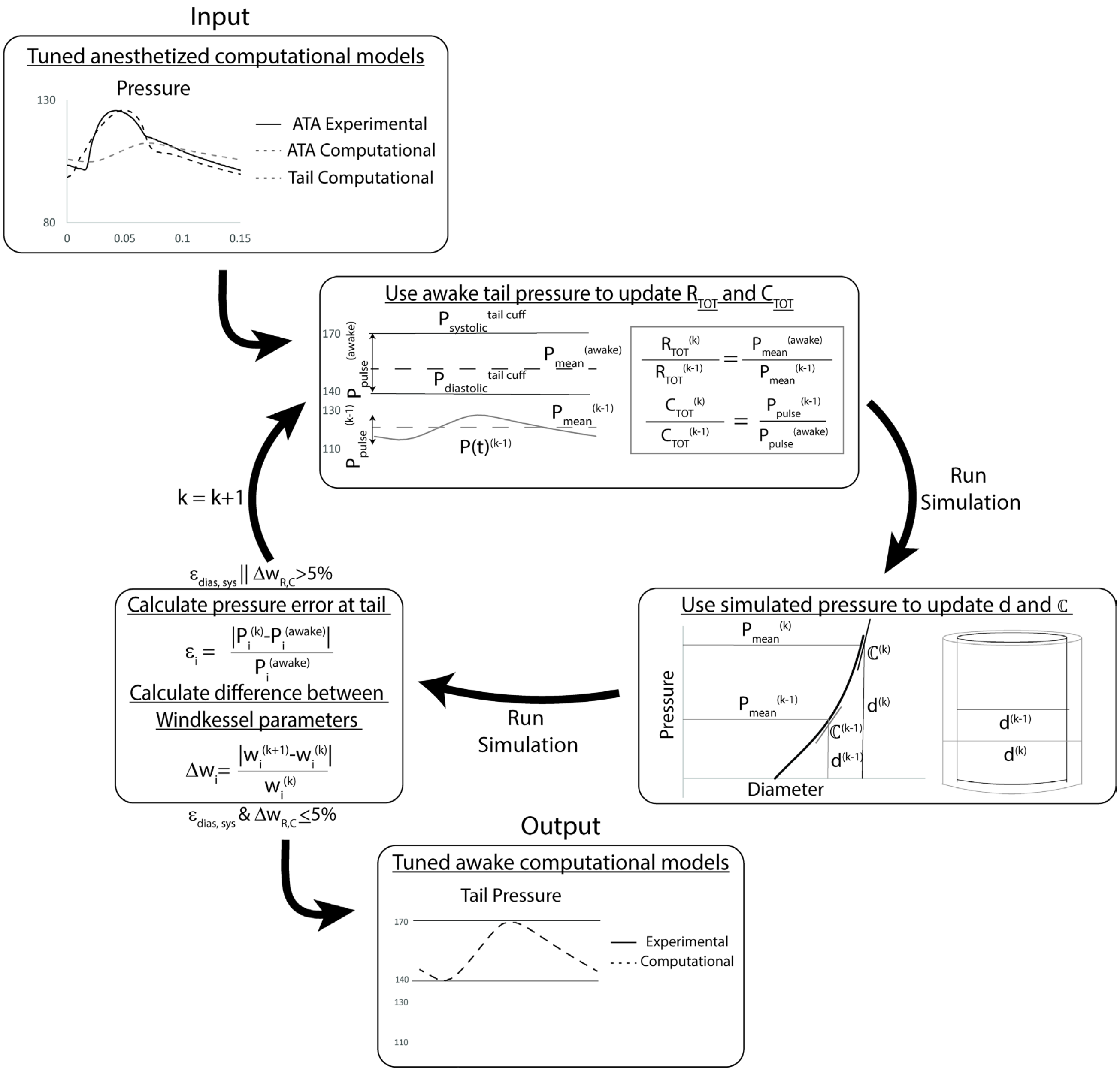


Figure 4-2. Iterative workflow to estimate awake vascular state from fully-calibrated anesthetized computational models.

Specifically:

Step 1 - updating R_{TOT} and C_{TOT}. The simulated outlet tail pressure of the awake model was compared with the experimentally measured tail-cuff pressure to calculate new values of R_{TOT} and C_{TOT}. R_{TOT} was calculated using Equation 4-3; for each iteration k, R_{TOT} was scaled using the ratio of awake mean pressure (estimated via Equation 4-1) to computational mean pressure. C_{TOT} was calculated using Equation 4-4; for each iteration k, C_{TOT} was scaled using the ratio of computational pulse pressure to awake pulse pressure (calculated via Equation 4-2). $C_{TOT}^{(k)}$ and $R_{TOT}^{(k)}$ were then distributed to the outlet branches using the same flow distributions as the anesthetized model (described above).

$$P_{mean}^{awake} = \frac{2}{3} P_{dias}^{tail\,cuff} + \frac{1}{3} P_{sys}^{tail\,cuff}$$

Equation 4-1. Estimation of mean pressure from diastolic and systolic pressure values.

$$P_{pulse}^{awake} = P_{sys}^{tail\,cuff} - P_{dias}^{tail\,cuff}$$

Equation 4-2. Calculation of pulse pressure from systolic and diastolic values.

$$R_{TOT}^{(k)} = \frac{P_{mean}^{awake}}{P_{mean}^{(k-1)}} R_{TOT}^{(k-1)}$$

Equation 4-3. Update of R_{TOT} based on ratio of awake to previous iteration mean pressure.

$$C_{TOT}^{(k)} = \frac{P_{pulse}^{(k-1)}}{P_{pulse}^{awake}} C_{TOT}^{(k-1)}$$

Equation 4-4. Update of C_{TOT} based on ratio of previous iteration to awake pulse pressure.

Computational simulations were then run with the new Windkessel parameters, leading to changes in P_{mean} throughout the vascular system.

Step 2 - updating d and $\mathbb{C}$. Pressure-diameter curves, reconstructed from mouse-specific parameters for the four-fiber family stored energy density function, were used to update the mean diameter $d^{(k)}$ corresponding to $P_{mean}^{(k)}$ for multiple vascular segments (ATA, pDTA, dDTA, SAA, IAA, right CCA, and left CCA). The 3D vascular model was then scaled linearly using the ratio of $d^{(k)}$ to $d^{(k-1)}$ for each segment. Updated values of vascular stiffness $\mathbb{C}^{(k)}$ for each segment were determined through linearization at

$(d^{(k)}, P_{mean}^{(k)})$. Wall thickness $h^{(k)}$ was calculated based on the assumption of incompressibility with the arterial wall modeled as locally cylindrical.

Step 3 - error minimization. Computational simulations were then run with new diameter, stiffness, and thickness values. The relative error $\varepsilon^{(k)}$ was calculated between computationally-determined and experimentally-measured systolic and diastolic tail pressures. The percent differences of the Windkessel parameters (Δw) were calculated based on $C_{TOT}^{(k)}$, $R_{TOT}^{(k)}$ and $C_{TOT}^{(k+1)}$, $R_{TOT}^{(k+1)}$. The awake computational model was considered to be fully calibrated when the stopping criteria of less than or equal to 5% was met for all error conditions: $\varepsilon_{dias}^{(k)}$, $\varepsilon_{sys}^{(k)}$, Δw_R, and Δw_C. Enforcing a low error yields a model representative of the awake hemodynamic conditions, whereas enforcing a small percent change in Windkessel parameters ensures a stable parameter space. To illustrate this process, the points on the pressure-diameter and resulting tail waveforms with R_{TOT}, C_{TOT}, ε_{sys}, and ε_{dias} for each iteration for one mouse (AngII - A1) can be seen in Figure 4-3.

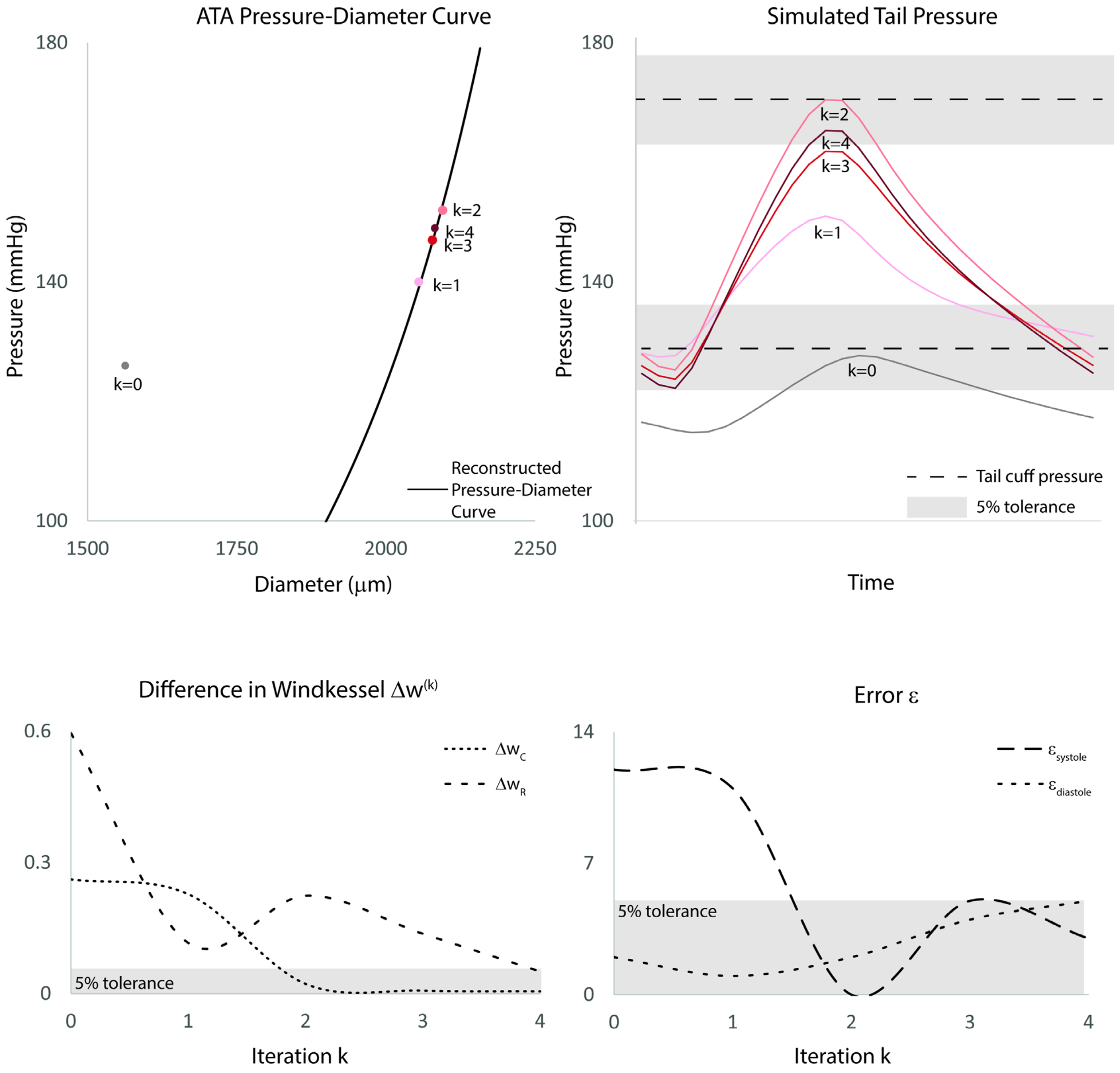


Figure 4-3. Iterative changes in computational parameters to estimate awake conditions for mouse A1 with k=0 being the tuned anesthetized model and k=4 being the tuned awake model. Top left: pressure-curve reconstructed from ex vivo *experimental parameters with* $(P^{(k)}, d^{(k)})n$. *Top right: tail outlet waveforms with awake systolic and diastolic measurements and 5% tolerance. Bottom left: differences in total resistance (dashed) and compliance (dotted) with 5% tolerance. Bottom right: relative error for systolic and diastolic tail pressures.*

4.3 Results

All AngII-infused mice used in this study exhibited some characteristics of the typical hypertensive phenotype, including increased systemic blood pressure. Further, mouse A1 presented an aortic dissection in the SAA and mouse A3 presented with aortic valve regurgitation causing diastolic backflow in the ATA.

4.3.1 Validation

Hemodynamics under anesthesia: Full multi-domain FSI simulations were first run for each of the six mice under the anesthetized conditions that were used to collect the ultrasound, microCT, and invasive pressure data. Whereas mouse-specific ATA flows were prescribed as inlet boundary conditions (Figure 4-4A), the computationally predicted values of ATA pressure and similarly CCA and IAA flows were then compared to experimental measurements (Figure 4-4B). In each case, predicted mean and pulse pressure in the ATA and mean flow in the CCA and IAA were within 10% of the measured values for all mice that had sufficient information for direct comparison. These validations provided confidence for implementing the aforementioned workflow for determining associated parameter values for awake conditions.

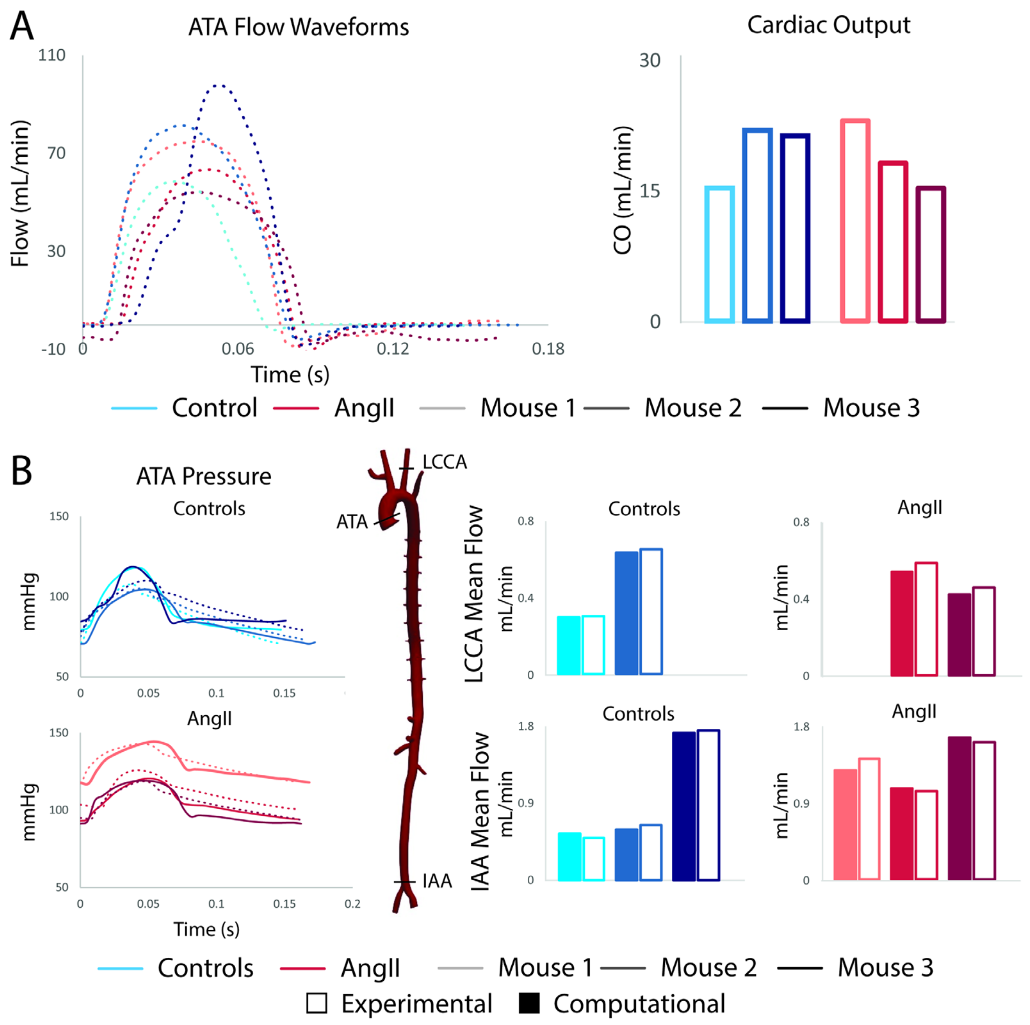


Figure 4-4. A. Imposed inlet boundary condition with flow waveforms (left) and cardiac output (right). B. Good agreement between anesthetized models and experimental data, shown with ATA pressure waveforms (left) and LCCA and IAA mean flows where available (right).

Hemodynamics under conscious conditions: Recall that the only hemodynamic information available in the awake mice was the tail-cuff pressure data. Regional values of luminal diameter, wall stiffness, and outlet boundary conditions (R_{TOT}, C_{TOT},) were thus tuned iteratively until the computed systolic and diastolic tail pressures matched experimentally measured tail pressures within 5% and with a stopping condition for R_{TOT} and C_{TOT} requiring the calculated value for the next iteration to differ by less than 5% from that for the prior iteration. The number of iterations k required for each mouse varied from 2 (control mouse C2) to 8 (control mouse C3). The good agreement between the computed and experimental systolic and diastolic tail pressure can be seen in Figure 4-5.

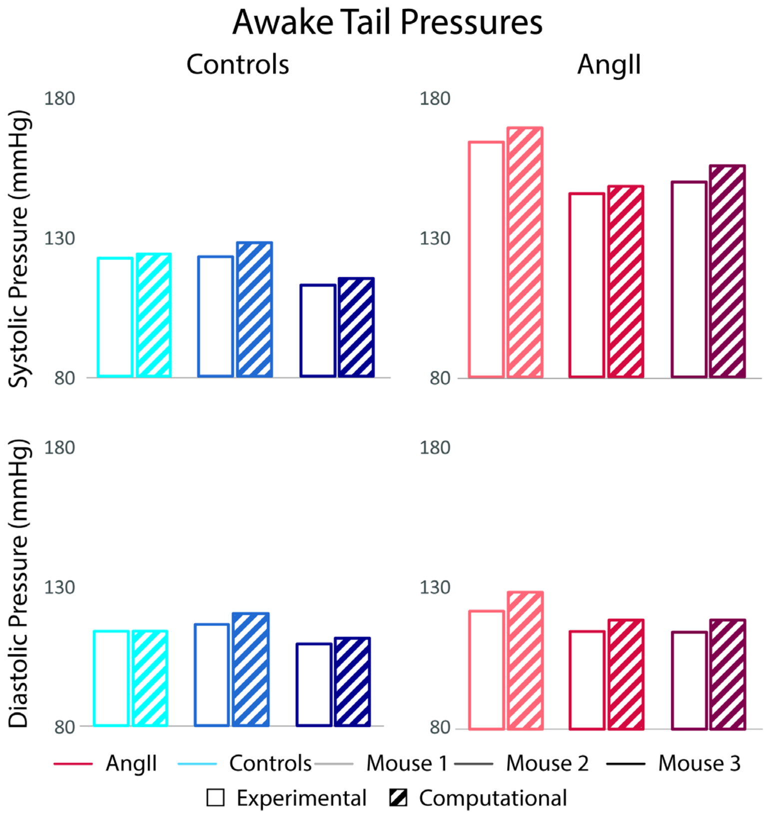


Figure 4-5. Computational (cross-hatched) and experimental (open) awake systolic (top) and diastolic (bottom) tail pressures were tuned to be within 5% difference.

4.3.2 Vascular structure

R_{TOT} and C_{TOT} can be separated into components based on contributions from central (3D computational domain) and peripheral (Windkessel parameters). Figure 4-6 shows the vascular resistance and compliance for each mouse studied separated into these components. Awake mice had larger resistances and smaller compliances than anesthetized mice, and these differences were larger in the AngII-infused mice than controls. For resistance, this difference was larger in the peripheral component than central. Awake AngII-infused mice had larger resistances and similar compliances than controls. For resistance, this difference was again larger in the peripheral component. For compliance, awake AngII-infused mice had smaller peripheral, but larger central compliances than controls, which accounts for the similar total compliances.

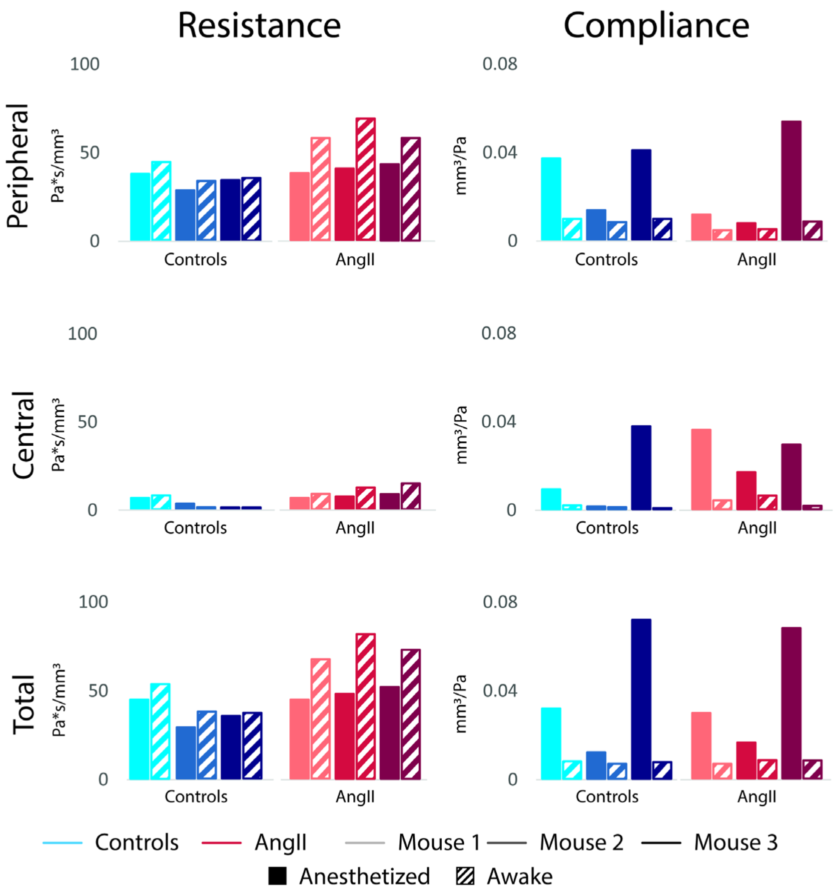


Figure 4-6. Peripheral (top), central (middle), and total (bottom) resistance (left) and compliance (right) for controls (blue) and AngII-infused (red) mice for both anesthetized (solid) and awake (cross-hatched) computational models.

Inner diameter was compared for five aortic regions and a CCA (Figure 4-7). Awake computational models have larger inner diameters than anesthetized for each aortic region. For both controls and AngII-infused, the increase was smaller in the IAA than in other aortic regions. AngII-infused mice again show larger changes with anesthesia than controls. Awake AngII-infused mice had larger inner diameters than controls in the abdominal aortic regions (ATA, pDTA, and dDTA) and CCA, while they were similar in the SAA and IAA, except in the SAA for mouse A3. The mouse-to-mouse variations in the effects of AngII-infusion can be appreciated in regard to inner diameter. In the ATA, pDTA, dDTA, and SAA, mouse A3 had larger diameters than the other two AngII-infused mice. However, inner diameters were similar between the three AngII-infused mice in the IAA and CCA. Although not shown, the AngII-infused mice also exhibited longer IAA regions than control mice.

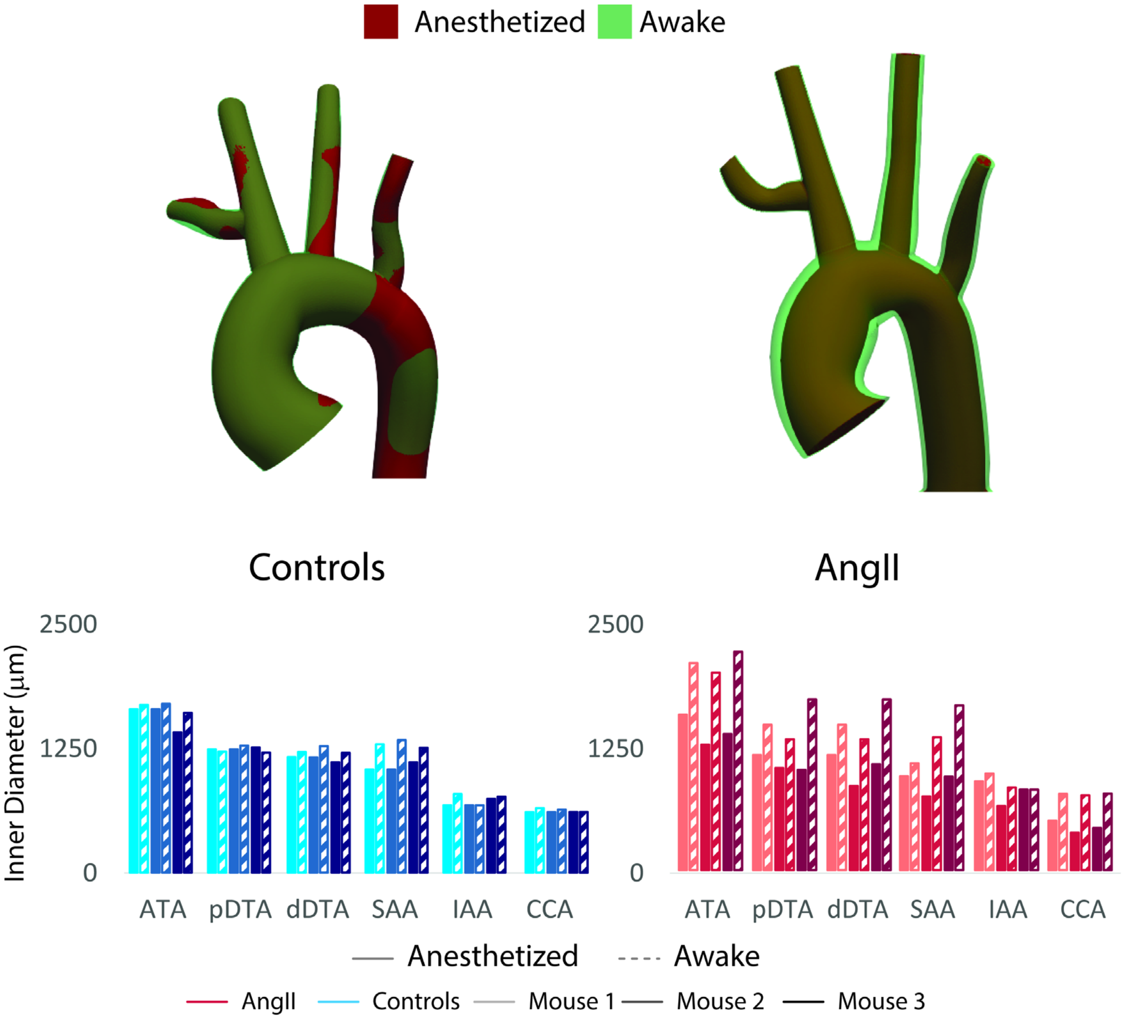


Figure 4-7. Inner diameter for anesthetized and awake models. Top: Upper portion of 3D computational domain for awake (green) and anesthetized (red) for representative control and AngII-infused mice. Bottom: Inner diameter for each aortic region and a CCA for anesthetized (solid) and awake (cross-hatched) models for each mouse.

Circumferential ($\mathbb{C}_{\theta\theta\theta\theta}$) and axial ($\mathbb{C}_{zzzz}$) stiffness, vascular thickness, and structural stiffness for each mouse for both anesthetized and awake conditions can be seen in Figure 4-8. Awake mice had larger circumferential, axial, and structural stiffnesses and smaller thicknesses than anesthetized. Again, this difference was larger in AngII-infused mice than controls, which led to stiffnesses being smaller than the controls in the anesthetized state. For controls, the increase with anesthesia was larger in the abdominal aorta (SAA and IAA) than thoracic aorta. For AngII-infused mice, the increase with anesthesia was larger in the pDTA and dDTA than other aortic regions. In the awake state, the AngII-infused mice in general exhibited larger circumferential, axial, and structural stiffness and smaller thickness values than controls, although there were both regional and mouse-specific differences in trends. The ATA of AngII-infused mice exhibited similar circumferential stiffness but larger thickness, leading to increased structural stiffness compared to controls. The pDTA, dDTA, and SAA had larger circumferential stiffness and thickness, which greatly increased structural stiffness compared to controls. The IAA and CCA had larger circumferential stiffness, but similar thicknesses, leading to similar structural stiffness with controls. In terms of mouse-to-mouse variations, mouse A2 has the smallest circumferential stiffness and largest thickness compared to the other mice, while mouse A3 has the largest circumferential and axial stiffness and smallest thickness compared to the other mice. Mouse A1 is in between mouse A2 and A3 for circumferential and axial stiffness and thickness, except for in the SAA, where it presented with a dissection. The best fit perivascular stiffness parameter (k_s) was found to be C1 – 1, C2 – 40, C3 – 1, A1 – 10, A2 – 1, A3 – 10 Pa/mm, while perivascular dampening parameter (c_s) was found to be C1 – 300, C2 – 100, C3 – 300, A1 – 200, A2 – 100, A3 – 500 Pa*s/mm.

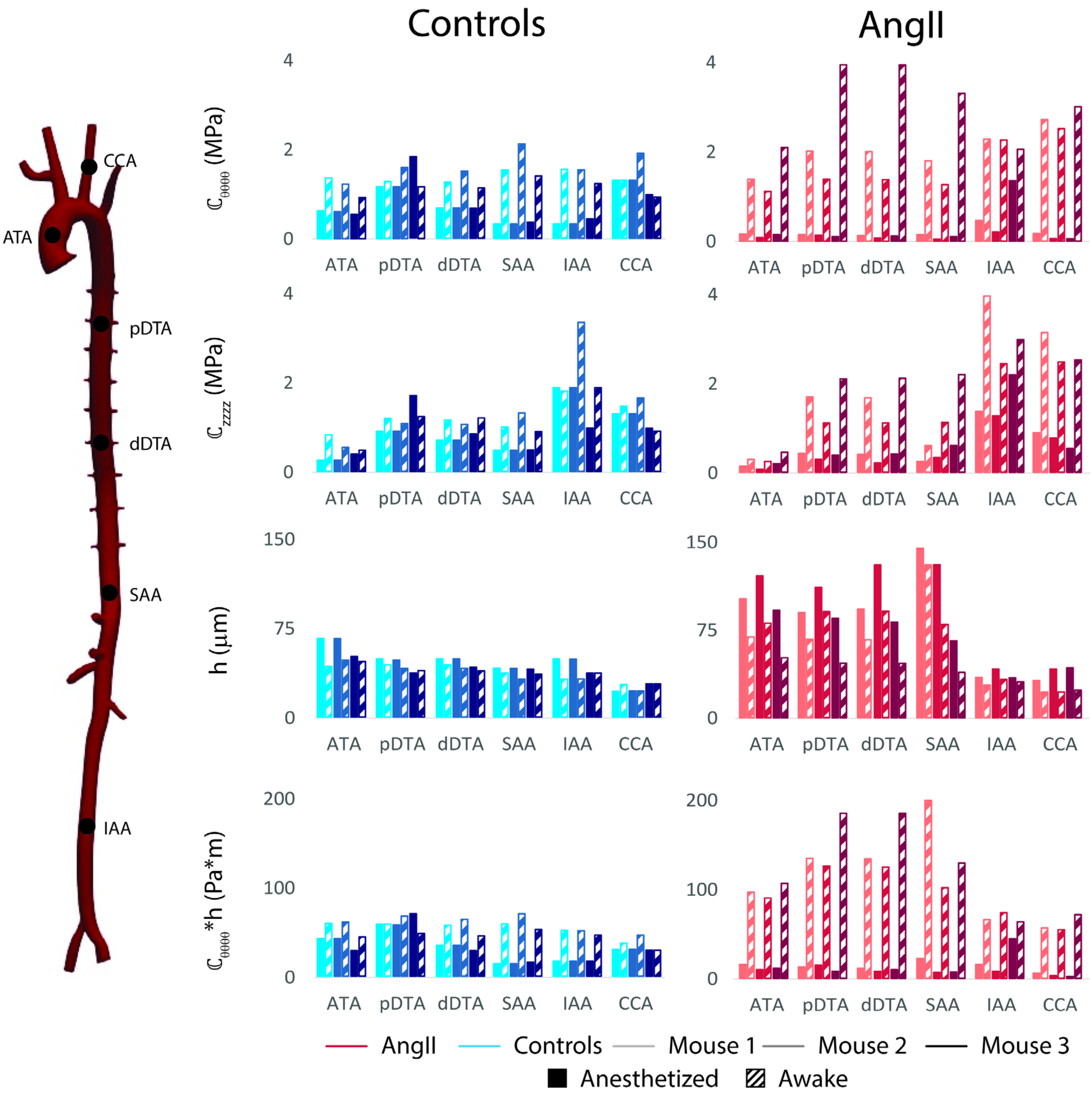


Figure 4-8. Vascular structure for each mouse under anesthetized (solid) and awake (cross-hatched) conditions.

Maladaptive vascular remodeling can be assessed by comparing the fold increase in systolic pressure ($\gamma = P/P_0$ where P_0 is the mean of control group systolic pressures) and the resulting fold increase in thickness ($\hbar = h/h_0$ where h_0 is the mean of control group thicknesses). If $\hbar$ is much larger than γ for a portion of the vasculature, remodeling is considered to be maladaptive for that region [124]. The values of γ and $\hbar$ for each AngII-infused mouse for the four regions of the vasculature and a CCA can be seen in Table 4-1. Mouse A1 exhibited severe maladaptive remodeling in the SAA, while mouse A2 exhibited maladaptive remodeling in all regions of the aorta except for the IAA. Mouse A3 exhibited no maladaptive remodeling, and conversely, had thickness ratios that were less than pressure ratios in all regions.

Table 4-1. fold increases in systolic pressure (γ*) and thickness (*$\hbar$*) with AngII-infused mice.*

	ATA		DTA		SAA		IAA		CCA	
	γ	$\hbar$	γ	$\hbar$	γ	$\hbar$	γ	$\hbar$	γ	$\hbar$
A1	1.34	1.5	1.43	1.6	1.41	3.6	1.42	0.8	1.36	0.8
A2	1.18	1.7	1.21	2.1	1.22	2.2	1.22	1.0	1.18	0.8
A3	1.24	1.1	1.30	1.1	1.30	1.1	1.29	0.9	1.24	0.9

4.3.3 Regional Hemodynamics

Pulsatile pressure and flow can be assessed at any location in the 3D computational domain, allowing for in depth examination of the effects of regional variations in vascular structure on local hemodynamics. Mean and pulse pressures can be seen in Figure 4-9A for each mouse for each region of the aorta, a CCA, and the tail for the anesthetized and awake computational models. Each mouse exhibited an increase in both pulse and mean pressure from the anesthetized to the awake. This increase was larger in the AngII-infused mice compared to control and in pulse pressure compared to mean pressure, which led to anesthetized pulse pressure being smaller in AngII-infused mice than controls. The change in mean pressure with anesthesia was similar in all aortic regions, whereas pulse pressure had larger changes in the abdominal regions (SAA and IAA) and tail than the thoracic regions (ATA, pDTA, and dDTA) and CCA for both control and AngII-infused mice. For the control mice, there was less variation between mice in mean and pulse pressure in the awake models than the anesthetized. Awake AngII-infused mice had larger mean pressures than controls in each of the regions analyzed. Mouse A1 had much larger pulse and mean pressures than the control mice, while mice A2 and A3 had similar pulse pressure to controls in the more proximal regions of the aorta (ATA and pDTA) and the CCA, and then larger in the other regions. Both controls and AngII-infused mice exhibited pulse attenuation down the aorta. This can be seen for each awake AngII-infused mouse compared to a representative control (C3) in Figure 4-9B. The pulse attenuation is smaller in the AngII-infused mice than controls. The difference in pressure between AngII-infused mice was mainly due to differences in systolic blood pressure, with diastolic pressures being more similar between AngII-infused mice.

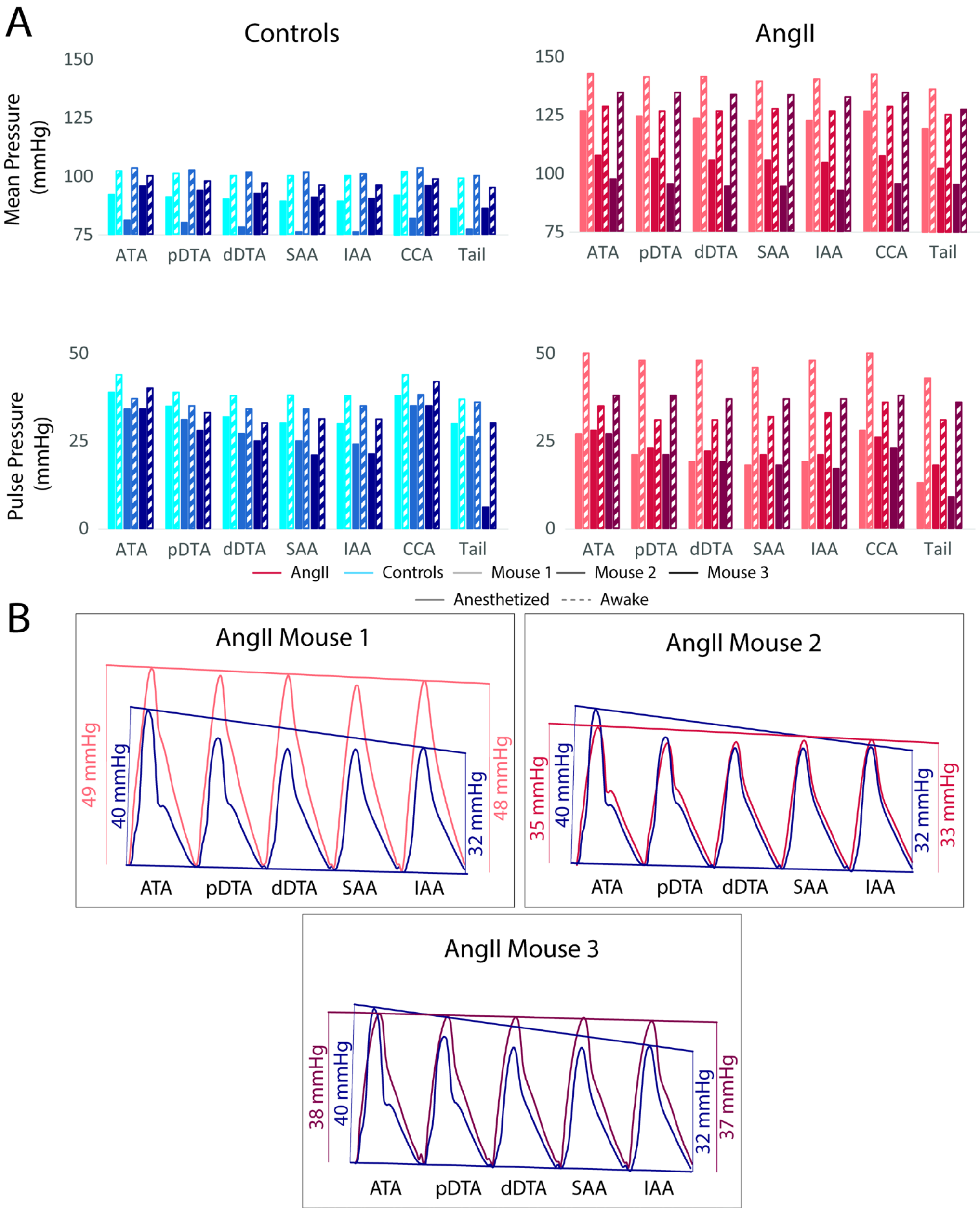


Figure 4-9. Effects of anesthesia and AngII-infusion on regional blood pressure. A. Mean and pulse pressure for 5 aortic regions, a CCA, and tail for anesthetized (solid) and awake (crosshatched). B. Pressure waveforms for 5 aortic regions for each awake AngII-infused mouse compared to C3 normalized to the same mean pressure and cardiac cycle length with ATA and IAA pulse pressure marked.

Flow waveforms are shown at 4 aortic sites (ATA, DTA, SAA, and IAA) for all six mice under both anesthetized and awake conditions (Figure 4-10). Mean flows were similar between AngII-infused and control mice in all aortic regions, except for the IAA, where AngII-infused mice have a markedly higher mean flow. AngII-infused mice also exhibited longer left ventricular ejection times (LVET; control mice C1 – 65 ms, C2 – 72 ms, C3 – 65 ms versus AngII-infused mice A1 – 71 ms, A2 – 71 ms, A3 – 75 ms). As mentioned previously, A3 exhibited aortic regurgitation causing increased backflow in the ATA.

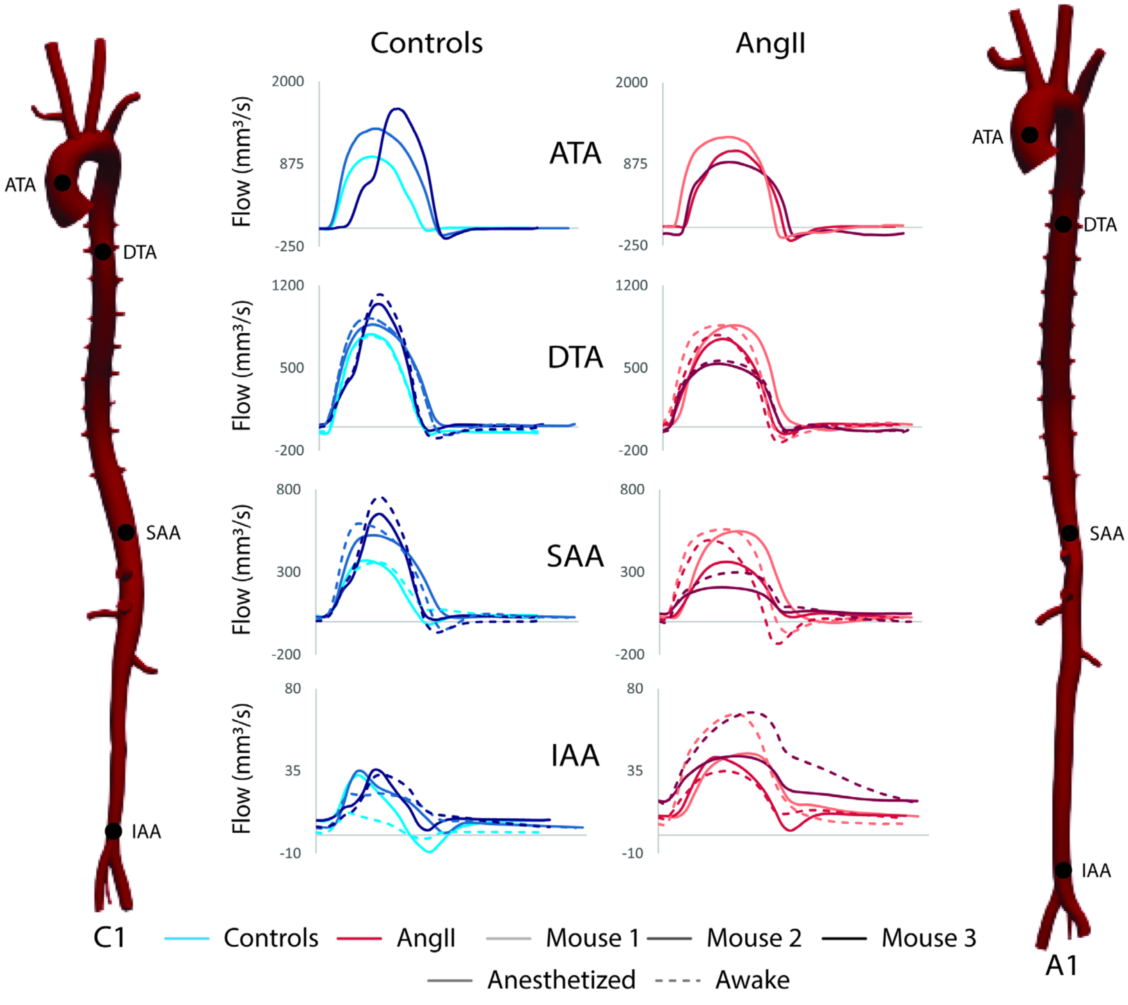


Figure 4-10. Flow waveforms for four locations in the aorta.

4.4 Discussion

During many forms of *in vivo* data collection from mice, anesthesia is necessary to ensure comfort of the animal and reduce noise associated with stress and motion. Relaxation associated with anesthesia decreases systemic pressure, vascular luminal diameter, and, because of the non-linear stress-strain relationship, stiffness. If the chosen anesthetic has similar effects on each region of the vasculature and each study cohort, trends from these studies are likely to not be affected by the use of anesthetic; however, previous studies have shown an inhibitory relationship between isoflurane anesthesia and AngII-induced vasoconstriction [117]–[119]. In this study, action research is used to improve the generalizability of murine studies with AngII-infused mice that require isoflurane anesthesia. In this section, you will find a discussion of the **effects of isoflurane anesthetic** on controls and AngII-infused mice, **effects of AngII-infusion** on *in vivo*, awake hemodynamics and vascular structure, **mouse-specific variations in hypertensive phenotype** from AngII-infusion, and **limitations and assumptions** of our workflow and experimental design. A discussion of the insights pertaining to future murine studies can be found in Chapter 5.

4.4.1 Effects of isoflurane anesthesia

Through comparison of anesthetized and estimated awake computational models, the impacts of isoflurane anesthesia on hemodynamics and structure of the central vasculature were analyzed in both control and AngII-infused mice. Many of the results are consistent with the known mechanisms of isoflurane. SVR decreased from the awake to the anesthetized state, especially in the periphery, which is consistent with the relaxation of vascular smooth muscle cells causing vasodilation with isoflurane [110]. Vascular compliance increased with anesthesia, which is also related to vasodilation and the pressure-dependent decrease in arterial stiffness. Isoflurane decreased mean pressure, because of decrease in SVR, and pulse pressure, because of increased compliance. Because of the decrease in mean pressure, vascular luminal diameter and stiffness decreased and thickness increased, which also contributes to the decrease in pulse pressure. Further, the *in vivo,* awake descriptors of vascular structure, including stiffnesses and thickness, are similar to other studies of C57BL/6 mice and AngII-infused C57BL/6 mice that linearize with awake pressure measurements [104], [125]. In the control mice, we identified spatially varying effects of isoflurane on aortic structure and hemodynamics. Stiffness decreased more in the abdominal aortic regions (SAA and IAA) than thoracic regions (ATA and DTA). This may be because of differences in mechanical properties from differences in elastin and collagen content. Further, the active relaxation of the vascular smooth muscle with anesthesia is not included in the simulations, which are especially important in the distal regions of the aorta. Further studies should further investigate this finding. Adjusting for anesthesia leads to less variation in mean and pulse pressure between control mice, which suggests that the workflow may be reducing the dose-dependent effects of anesthesia caused by difficulty to ensure equal doses with inhalant anesthetics [64].

We identified differential effects of isoflurane anesthesia on AngII-infused mice, which is consistent with previous studies [116]–[119]. To our knowledge, this is the first characterization of the effects of acute isoflurane use in chronic AngII-infused mice on global hemodynamics and vascular structure. Isoflurane decreased systemic mean and pulse pressure more in AngII-infused mice than controls (Figure 4-11). This caused the pressure-dependent properties of the vascular wall to also be depressed more in AngII-infused mice. Importantly, this caused AngII-infused mice to have lower pulse pressure and vascular stiffness than control mice while under anesthesia, which defied *ex vivo* biomechanical findings and expected effects of AngII-infusion. While we are unable to examine the mechanisms of this interaction with the computational workflow, the larger decrease in peripheral resistance in AngII-infused mice than controls with anesthesia is consistent with the hypothesis that isoflurane reduces the ability of AngII to induce vasoconstriction in vascular smooth muscle cells [117], [119]. Furthermore, the relationship between AngII and isoflurane may relate to the increased risk of intraoperative hypotension observed in patients with chronic systemic hypertension or control their blood pressure using RAAS agonists, which should be further explored [126]–[128].

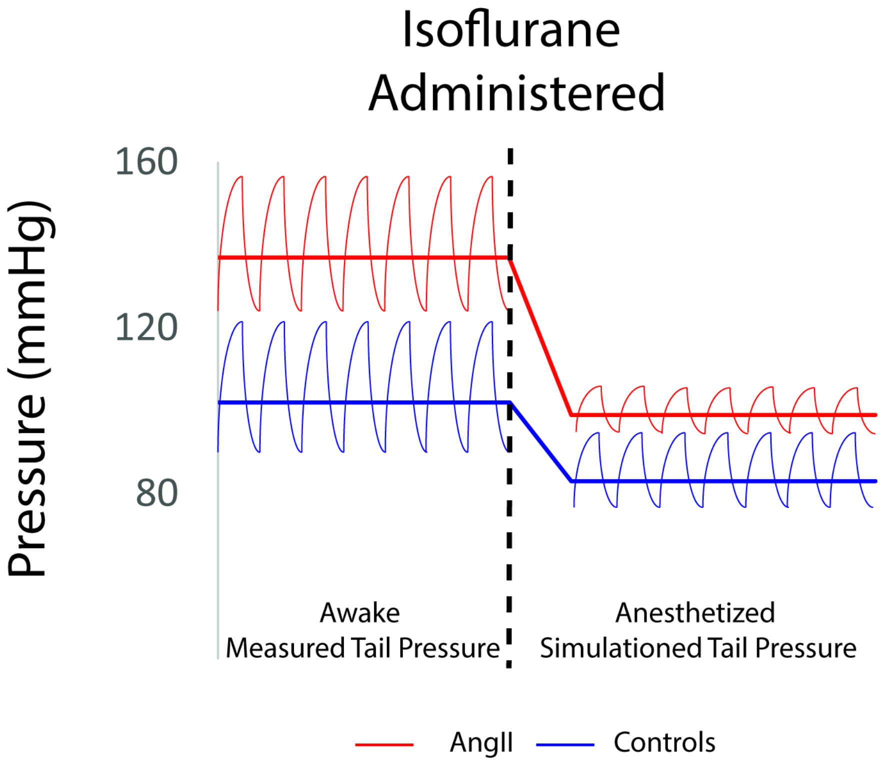


Figure 4-11. Visualization of the effects of acute isoflurane administration on tail pressure of control and AngII-infused mice created from average anesthetized (computational) and awake (experimental) mean and pulse tail pressure of control (blue) and AngII-infused (red) mice.

4.4.2 Effects of AngII-infusion

Through comparison between AngII-infused and controls awake computational models, we examined the effects of chronic AngII-infusion on *in vivo* vascular structure and hemodynamics. In this section, results are generalized between the three AngII-infused mice, while a discussion of mouse-to-mouse variation can be found below. AngII-infused mice had larger vascular resistance than controls, most of which is in the peripheral component. This is consistent with increased vasoconstriction with AngII-infusion. Luminal diameters are slightly larger in AngII-infused mice, and more so in the abdominal aorta (ATA, pDTA, and dDTA) and CCA. Regional differences in effects of AngII-infusion are consistent with Bersi *et al.* (2017): the ATA exhibited slight increases in circumferential stiffness, decreases in axial stiffness, and increased thickness; the DTA and SAA exhibited increases in circumferential stiffness, axial stiffness, and thickness; and the IAA exhibited slightly increased circumferential stiffness, increased axial stiffness, and similar thicknesses [97]. We also characterized the CCAs, which, as expected due to similar function and properties, has similar effects as the IAA.

The value of computational analysis in this case is the characterization of local hemodynamics in response to the spatially varying vascular stiffening with AngII-infusion. Because of increases in vascular resistance, mean pressure increased with AngII-infusion and this increase was similar for all aortic regions. On the other hand, differences in pulse pressure were region specific. For most AngII-infused mice, pulse pressure is similar to controls in the ATA and CCA. Pulse pressure increases in the more distal aortic regions, because of large increases in structural stiffness in the DTA and SAA. This is a likely a leading contributor to the propensity of AngII-infused mice to exhibit dissections in the SAA [97], like mouse A1. This differential increase in pulse pressure decreases pulse attenuation down the aorta, which differs from the naturally aged mouse phenotype seen in Chapter 3. Interestingly, we characterized less remodeling in the IAA compared to other regions, similar to [97], including with a smaller increase in diameter, thickness, and structural stiffness; however, we still found an increase in pulse pressure in this region compared to other regions, which may be attributed to the increase in mean flow in this region. Future studies should examine the impact of increased distal pulse pressure on end-organ damage in the abdominal and renal capillary beds. Finally, we observed that AngII-infused mice exhibited increased left ventricular ejection times, which may be an indicator of cardiac remodeling in response to the increase in afterload associated with hypertension [129]. This finding may be an early sign of ventricular hypertrophy, as AngII-infusion is common mouse model for studying diastolic dysfunction and heart failure with preserved ejection fraction (HFpEF) [130]–[134], although sufficient experimental data pertaining to cardiac function was not collected to corroborate this. Future studies could employ a heart model [69], [70] or couple 3D ventricular models to the arterial domain [135] to better study cardiac function and HFpEF disease progression.

4.4.3 Mouse-specific variations in hypertensive phenotype

Since all experimental data was collected from each individual mouse used in this study, computational models presented are truly mouse-specific, which allows us to analyze the mouse-to-mouse variations in the effects of AngII-infusion. The following discussion uses the awake computational models to analyze these differences between mice. Table 4-2 summarizes the generalized trends in computational and experimental metrics for each AngII-infused mouse in relation to the other AngII-infused mice. Mouse A1 had medium values for diameter, stiffness, and thickness with some degree of maladaptive remodeling and largest CO, mean pressure, and pulse pressure. The increase in mean pressure compared to the other mice is likely due to increased CO, as it had the smallest R_{TOT}. An increased CO also implies an increased stroke volume (SV) because mice had similar heart rates, as seen by similar cardiac cycle lengths. The larger pulse pressure observed in this mouse, which was over 10 mmHg larger than the other mice, is likely due to the increase in SV, as it did not have the largest structural stiffness compared to the other mice. The increase in mean pressure compared to the other mice is also likely due to CO, as it had the smallest R_{TOT}. Additionally, mouse A1 had an aortic dissection in the SAA, which was likely observed because of the much larger pulse pressures.

*Table 4-2. Ranking of each AngII-infused mice in comparison to the other AngII-infused mice. *,**, and *** and green, orange, and red shading correspond to smallest, medium, and largest values.*

	Metric	Reference	A1	A2	A3
Vascular Structure	Diameter	Figure 1-6	**	*	***
	$\mathbb{C}_{\theta\theta\theta\theta}$	Figure 1-7	**	*	***
	$\mathbb{C}_{zzzz}$	Figure 1-7	**	*	***
	h	Figure 1-7	**	***	*
	Cqqqq*h	Figure 1-7	**	*	***
	Maladaptive modeling	Table 1-1	**	***	*
	R_{TOT}	Figure 1-5	*	***	**
	$R_{peripheral}$	Figure 1-5	*	***	**
	$R_{central}$	Figure 1-5	*	***	**
	C_{TOT}	Figure 1-5	*	***	**
	$C_{peripheral}$	Figure 1-5	*	**	***
	$C_{central}$	Figure 1-5	**	***	*
Hemodynamics	CO	Figure 1-3	***	**	*
	Central Mean Pressure	Figure 1-8	***	*	**
	Central Pulse Pressure	Figure 1-8	***	*	**
	LVET	Figure 1-9	** +	** +	***
	Additional Considerations		SAA dissection		Aortic Regurgitation

+ mice A1 and A2 had the same LVET value

Mouse A2 had the smallest values for circumferential and axial stiffnesses, but the largest thickness and maladaptive remodeling. This suggests that for this mouse, vessel thickness was increased in a way that had little effect on the material stiffness of the vessel. This phenotype is consistent with Korneva and Humphrey's (2019) description of AngII-infused mice with maladaptive modeling [136]. Because Korneva and Humphrey (2019) also related high degrees of maladaptive remodeling with an impairment of smooth muscle cells to constrict, it is possible that mouse A2 exhibited this impairment, although sufficient data to confirm this was not collected. Although not shown, mouse A2 had the smallest *in vivo* axial stretch of the AngII-infused mice, which was also exhibited in the maladaptive mouse phenotype in [136]. This mouse also exhibited the largest increase in all components of resistance. The largest increase in R_{TOT} is due to the smallest diameter values. The increase in $R_{periphery}$ suggests an increased remodeling in the periphery as well, although future studies would need to confirm this. Despite the evidence of maladaptive remodeling in this mouse, it had the lowest mean and pulse pressures, likely related to the lesser increase in CO than mouse A1 and smaller structural stiffness than A3.

Mouse A3 exhibited the largest increase in material stiffness, in both the circumferential and axial directions, but with the smallest thickness of the AngII-infused mice, suggesting that vascular remodeling had a larger impact on material stiffness than thickness. The large increases in material stiffness led to larger structural stiffnesses than the other mice, despite the smallest degree of vascular thickening and no maladaptive modeling. This mouse had the smallest CO, largest LVET, and aortic regurgitation resulting in increased diastolic backflow in the ATA. This may indicate a further progression of cardiac dysfunction compared to the other mice, but this would need to be validated with further experimental procedures.

In summary, mouse-specific computational modeling allows for in-depth analysis of the variations in hypertensive phenotype with AngII-infusion. For mouse A1, the increase in CO and SV can explain many of its hypertensive phenotype, including increased pulse pressure and mean pressure, observed. Mouse A2 exhibited maladaptive remodeling, which increased structural stiffness through increasing vessel thickness but without changing material stiffness. Conversely, mouse A3 had increased material stiffness but with very little thickening. These different phenotypes suggest different mechanisms of excess circulating AngII, although the causes of these differences need to be examined in the future.

4.4.4 Limitations and assumptions

There are multiple limitations and assumptions in the presented study both in experimental design and computational workflow to adjust for the effects of anesthesia. The largest limitation to this study is the small sample population. The insights discussed herein, especially concerning the effects of AngII-infusion, need to be further verified with a larger mouse population. Further, although completing all experimental procedures on each mouse allowed for the creation of mouse-specific computational models, we needed to assume that experimental conditions were the same for each procedure, including that depth of anesthesia. It is also assumed that the preceding experiments did not alter hemodynamics and structure, which may not be the case given the stress put on the animals and potential for vascular damage from the invasive Millar catheterization.

For the workflow to estimate awake conditions, we assume that there are no changes in flow distribution from the aorta with isoflurane anesthesia. Previous studies have reported that there might be some alterations to preserve cerebral blood flow [107]. Further, we assume that there is no change to the ratio of distal to peripheral resistances at each outlet. Importantly, the use of tail cuff pressure as the only awake pressure measurements leads to experimental variability. As mentioned in Chapter 2, the stress of being handled during blood pressure cuff assessment can increase blood pressure and heart rate [137]. In this study, mice were preconditioned to the measurement apparatus for several days before measurements were taken. There are multiple catheter-based methods for continuous recording of blood pressure in awake mice, including implanted telemetry [138]. While these are able to more closely monitor central pressures in the awake state and obtain pressure waveforms, the addition of the catheter affects the hemodynamics of the system [89] and may induce inflammation and remodeling. For these reasons, we have decided to use tail cuff measurements for assessment of awake pressure, although further studies could be performed to assess the differences in estimated awake hemodynamics between awake pressure measurement techniques.

In summary, we presented a new workflow that enables researchers to simulate central hemodynamics in mice under awake conditions, based in part on detailed measurements that are available only under anesthetized conditions. We showed that differential effects of anesthesia on different groups of mice can confound simulated results and their interpretation. Accounting for these differential effects in AngII-infused and non-infused *C57BL/6J* mice, revealed that *in vivo* hemodynamics are estimated poorly in the AngII-infused mice in the absence of appropriate understanding of the effects of isoflurane anesthesia. Finally, the awake computational models allowed us to characterize better the increases in *in vivo* vascular stiffness particularly in the DTA and SAA with AngII infusion, which increases pulse pressure throughout the aorta and decreases the typical pulse pressure attenuation in the abdominal segment. Having local hemodynamics, both pressure and flow, promise to help us to understand better previously reported results on regional differences in aortic adaptation or maladaptation during AngII-induced hypertension [97] (Bersi *et al.*, 2017), thus emphasizing the importance of coupling computational and experimental studies as well as *in vivo* and *ex vivo* measurements. Finally, given the marked specimen-to-specimen variability in AngII-induced pathologies [139], collecting all data from each mice will be critical for understanding differential disease progression.

Chapter 5 Discussion of Murine Experiments for Hypertension Research

5.1 Discussion

Hypertension is a highly prevalent cardiovascular disease and leading contributor to death worldwide [17]. Through a positive feedback relationship with arterial stiffness, hypertension causes end-organ-damage promoting the development of secondary diseases, including in the heart, brain, and kidneys [21], [22]. A better understanding of the relationship between hypertension, arterial stiffness, and progression of resulting pathology may be beneficial for development of treatment strategies and prognostic criteria; however, it is difficult to obtain the high-resolution, direct measurements necessary from human subjects *in vivo*. Murine models are commonly used to study hypertension and arterial stiffness because of their low cost, high availability of genetic manipulation, and short lifespans [26], [58]. Careful experimental design is necessary to ensure the results of murine experiments are transferable to human disease. Technical action research can be used to make decisions concerning experimental factors, including disease models, environmental conditions, and measurement techniques, to optimize the generalizability of results. In this work, we used FSI modeling of the cardiovascular system to obtain high resolution metrics of hemodynamics and vascular structure throughout the central vascular system, which allowed us to determine the impact of experimental decisions.

Aging is a primary risk factor for many cardiovascular diseases, including hypertension, and is, therefore, a common area of research for murine experiments. Multiple murine models have been employed to study cardiovascular aging, although differences between these models and natural human aging are often not considered in depth. In Chapter 3, we identified key similarities and differences in hemodynamics and vascular structure between young and old representative humans and mice through in-depth comparison of computational models of the central vasculature system. Human computational models were built from subject specific data on anatomy, hemodynamics, and vascular deformations and population specific data for parameters of the four-fiber family model for vascular mechanics. Trends observed with aging for the human subjects are consistent in many ways with expectations from literature. Murine computational models were built from population-based data on anatomy, hemodynamics, and vascular mechanics using allometric scaling as described in [48]. An in depth comparison between our results and previously published work can be found in Chapter 3 – Discussion. The implications of identified similarities and differences for improving generalizability of murine models for cardiovascular aging research are presented in section 1.1.

Measuring *in vivo* cardiovascular parameters in mice requires anesthesia to reduce discomfort and movement artifacts. Use of anesthesia should be carefully controlled, as it depresses cardiovascular function. Isoflurane is typically preferred for these experiments, because of an ease of controlling depth and quick recovery time [64]. Previous studies have reported a potential inhibitory mechanism of isoflurane anesthesia on angiotensin-II (AngII), a commonly used molecule for inducing hypertension in murine studies [116]–[119], [140]. In Chapter 4, the impact of isoflurane anesthesia on chronically AngII-infused mice was investigated through the development of a computational workflow for estimating awake hemodynamic conditions. The estimated awake parameters for hemodynamics and vascular structure are consistent with the expected effects of AngII-infusion, demonstrated through global increases in pressure and regional aortic remodeling similar to prior studies [97], [136]. Resulting insights pertaining to the effects of isoflurane anesthesia and chronic AngII-infusion can be found in Chapter 4 – Discussion.

Suggestions for future murine studies that aim to characterize *in vivo* hemodynamics of AngII-infused mice are presented in section 1.2.

5.2 Murine Models for Cardiovascular Aging

The identified similarities and differences between naturally aged humans and mice can be used to inform the appropriate context for using the naturally aged *C57BL/6* x *129/SvEv* mouse for cardiovascular aging studies. Regional similarities between the species indicate that the naturally aged *C57BL/6* x *129/SvEv* mouse may be appropriate for investigating the effects of aging on particular aortic regions. For example, both species exhibit a similar increase in pulse pressure and vascular stiffness in the ascending thoracic aorta (ATA). If a researcher is interested in studying this region in particular, this might be an appropriate mouse model. Furthermore, this may indicate that the age-related effects on cardiac function and coronary artery perfusion may be similar between species, although future studies should validate this. Regional differences between the species indicate for which regions the naturally aged *C57BL/6* x *129/SvEv* mouse is not an appropriate model. For example, the patterns of pulse pressure, vascular stiffness, and diameter tapering vary between species in the abdominal aortic regions. If a researcher is interested in studying the effects of aging in this region, this may not be an appropriate mouse model. Similarly, this may indicate that this is not an appropriate mouse model for investigating the effects of aging on, say, kidney perfusion. Furthermore, the differences in age-related changes in peripheral resistance and compliance between species may indicate that this mouse model is not appropriate for studying the effects of aging on distal vascular perfusion.

The in depth comparison between human and murine natural aging also informs the potential targets for the development of novel murine models for cardiovascular aging. A key difference between mechanisms of aging between species is the lack of elastin degradation in the murine aorta. Because of this, mouse models for cardiovascular aging have been developed that target elastin structure, including infusion of elastases and manipulation of key genes for elastin synthesis, including fibulin-5 (Fbln5). The FSI modeling techniques used in this work have been employed previously for a Fbln5 knock out mouse model (*Fbln5*$^{-/-}$) and similar patterns of aortic stiffening and pulse pressure were observed to the naturally aged mouse model presented herein [48]. To observe patterns of aortic stiffness and hemodynamics similar to humans, mouse models may need to incorporate differential stiffening of the distal aortic regions, which would induce an increasing stiffness gradient down the aorta. This may be possible because of the differences in structure between thoracic and abdominal aortic regions, although future work would need to be performed to identify potential mechanisms to achieve this. Aortic banding at level of the diaphragm may induce inflammation and increase abdominal stiffness locally as well.

In this work, we completed one action research cycle for improving the generalizability of murine results for cardiovascular aging, which lead to suggestions for appropriate use of the naturally aged *C57BL/6* x *129/SvEv* mouse model and potential targets for the generation of novel models for studying cardiovascular aging. Further iterations of the action research cycle should be completed based on these suggested improvements to improve the generalizability of murine experimental methods for studying cardiovascular aging. One potential direction for future action research cycles is to continue to assess the naturally aged *C57BL/6* x *129/SvEv* mouse model to further identify aspects of cardiovascular aging that are similar to humans. This could include examining alterations in cardiac function and organ perfusion with age. Another potential direction for future action research cycles is to apply FSI modeling techniques for additional models of cardiovascular aging to identify more appropriate mouse models. This could include models of elastin degradation and aortic stiffening. Researchers may also consider assessing other animal models, including other commonly used animal models for cardiovascular research such as rats or pigs, to determine if there are species that exhibit natural aortic aging more similar to the humans.

5.3 Anesthesia with AngII-infused Mice

Through a novel computational workflow to estimate awake hemodynamic conditions, we found that isoflurane anesthesia had a larger effect on chronically AngII-infused mice compared to controls. This suggests that researchers should consider using alternative anesthetics for studies with AngII-infused mice. Although not preferred by researchers because of long recovery times, ketamine is a potential alternative that should be explored, as other volatile anesthetics may have similar effects on AngII-infused mice [64], [118], [119]. The possibility of reducing the amount of anesthesia given to AngII-infused mice should also be considered, although the effects of AngII-infusion on the analgesic properties of isoflurane would need to be investigated to justify this. If alterations to experimental conditions are not feasible, the computational workflow presented in Chapter 4 may be used to adjust for the differential effects of anesthesia for these studies. In this case, future work should be performed to further validate the estimated awake hemodynamics with experimental data. Telemetry to assess awake blood pressure at various regions of the central vasculature could be used to achieve this. Researchers should also be aware of the necessary experimental data needed to inform computational models if they intend to use the workflow to estimate awake hemodynamic conditions. Of note, *ex vivo* pressure-diameter curves from biaxial tissue testing may diminish the ability of researchers to employ this method. Future work should also explore the impact of the constitutive relationship for aortic biomechanics on estimates of awake stiffness.

As with section 1.1, we completed one action research cycle for improving the generalizability of results from murine experiments that use isoflurane anesthesia with AngII-infused mice. Future iterations of the action research cycle should be completed based on the suggested improvements, which would lead to the optimization of experimental conditions for characterizing *in vivo* hemodynamics of AngII-infused mice. One continuation of this action research cycle would be to use the methods presented herein to explore the impact of different types of anesthesia. Researchers could also iterate on the dose of isoflurane used with AngII-infused mice, which would need to include metrics for consciousness and analgesia. Finally, the computational workflow developed could be optimized to future ensure accuracy of results and ease of use.

Chapter 6 Introduction to Interventions for STEM Education

6.1 Motivation

Many science, technology, engineering, and math (STEM) classrooms utilize an instructional paradigm for education, which focuses on the transfer of knowledge to students in a traditional lecturing format. However, this has been previously shown to be ineffective for the development of usable knowledge, which is essential for practical STEM careers. To better prepare STEM undergraduate students, student-centered pedagogies should be implemented, which focus on the act of learning by students rather than the transfer of knowledge from instructors. Practical action research can be used to optimize the implementation of student-centered pedagogies in the classroom to improve student outcomes. Another systemic issue in STEM education is a chilly climate for historically underrepresented groups, which is a significant barrier to success for individuals from these groups. Many diversity, equity, and inclusion (DEI) efforts in STEM education do not attempt to address the cultural discourses that promote this chilly climate and, therefore, have limited widespread success. Critical action research can be used to improve the climate in STEM for students through the development of interventions to dismantle the forces in STEM culture that cause DEI issues.

I am additionally motivated in this section because of my personal experiences with STEM education. Throughout my own undergraduate and graduate education, I was often frustrated by the teaching methods used in my classes. Many of my professors were focused more on the content of the lectures rather than the learning of students. Anecdotally, these classes did not help me become a confident biomedical engineer; my time outside of class was spent struggling to teach myself the material or to complete exercises only tangentially related to what would be included in assessments. I grew frustrated with my own abilities and struggled to see myself as an engineer. Even now, I struggle with confidence and skills covered in these content-focused, lecture-based classes. In contrast, I still remember content and have fond memories from classes in which professors actively engaged students with the content. It was clear that these professors cared about student learning, implemented research-based teaching strategies, and adapted to student feedback. Moving into a teaching focused position post-graduation, I hope to ensure that the teaching methods I use in my classroom are optimized for student learning.

I additionally struggle to identify as an engineer because I differ in many ways from the dominant cultural narrative of who an engineer should be, i.e., a person who has unrelenting focus pertaining to facts and technology, who does not struggle with any scientific or mathematical concept, who can dedicate 12 hours a day for work without break, and who does not care about feelings or non-technical skills. Many engineers who fall outside of this dominant narrative, for example because of cultural norms or neurodivergences, struggle to build engineering identity and to persist in the field. In addition, those with historically marginalized identities struggle in academia and the engineering field because of factors like lack of representation, under preparedness for higher education, less time to dedicate to coursework outside of the classroom, social stigma, and non-inclusive teaching practices. Colleges and universities undertake diversity, equity, and inclusion (DEI) efforts to help mitigate these factors and increase retainment and retention of diverse students, including initiatives and events to educate about identity-based issues, develop communities of support, provide resources, etc. Despite good intentions, many of these efforts do not have a large impact because of lack of support from those with power, misunderstanding of the needs of communities, under funding, etc. From a student perspective, many DEI efforts from universities feel disingenuous and misguided, because they are often not informed by the societal factors that lead to inequality or do not consider lived experiences of individuals within communities. I would like to help improve the climate in engineering and academia for those with marginalized identities and ensure that anyone who is interested in engineering has the proper support and resources to succeed through DEI efforts informed by the voices and needs of individuals from historically marginalized communities.

In this work, I demonstrate the application of practical action research to the improvement of instructional strategies for teaching computer programming to biomedical engineering students (Aim 3) and critical action research to the improvement of the climate in STEM for LGBTQIA+ individuals (Aim 4). A social-psychological framework is employed for evaluating these interventions, which emphasizes the importance of interpersonal and intrapersonal factors for the development of knowledge [141]–[144]. In this chapter, background information is provided related to social-psychological theory, student-centered learning, the cultural climate in STEM, and educational data collection strategies.

6.2 Social-Psychological Theory

As applied to education, social-psychological theory describes the influence of social and intrapersonal factors on student learning [145]. With a social-psychological lens, we situate the learner in their environment and acknowledge the importance of context for learning. The learner is both impacted by and influences their environment, and these relationships inform their academic behavior, which can be seen in Figure 6-1 [145]. For example, if a student is particularly interested in computer programming, they may situate themselves in environments that allow them to explore this field further. If an instructor complements their effort and skills in this area, they then develop confidence in their abilities, continue to pursuit their interests, and align their career goals with computer programming. Contrarily, if a student is unable to see themselves portrayed in the dominant narrative of an engineer, they may enter into higher education with already low confidence in their abilities despite interest and capabilities in the field. If they then do not feel a sense of belonging in engineering and academic environments, their interests may diminish, and their confidence decreases further. This may lead to them not setting academic goals, having low emotional well-being, and reducing the amount of effort they put towards academics, limiting their ability to succeed.

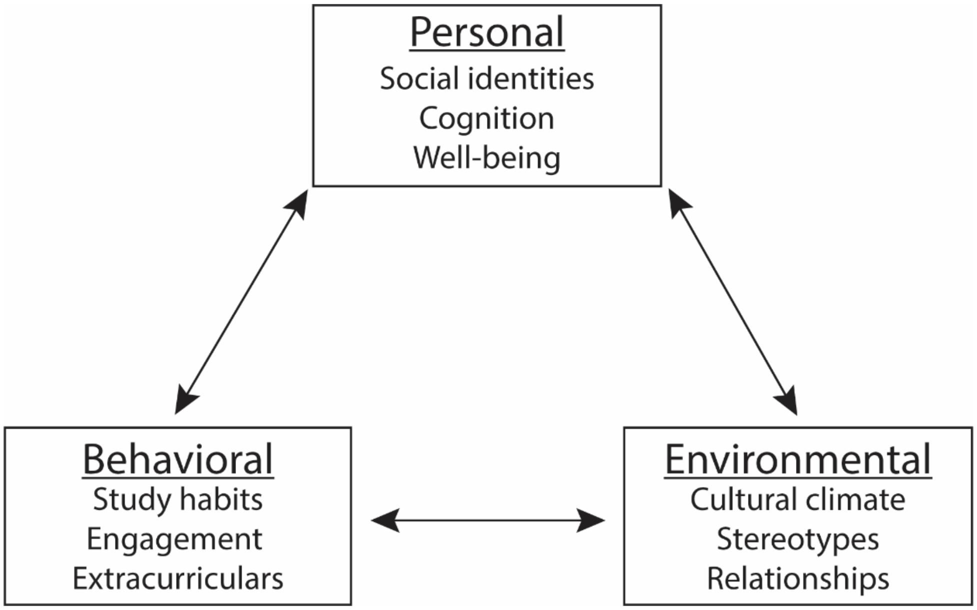


Figure 6-1. Social-Psychological Theory for higher education adapted from [146], [147].

Social-psychological indicators allow us to explore individuals' relationships with their environments and the effects of these relationships on the individual, including through sense of belonging, community, motivation, self-efficacy, engagement, and identity. These indicators are dynamic, interconnected, and have been shown to affect metrics of learning and success in STEM, including GPA and retention [142], [144], [147]–[151]. Therefore, interventions can be developed that aim to increase these factors to improve student outcomes, and these factors can be used to assess the impact of various interventions. In this work, social-psychological factors were used to assess the impact of the developed interventions during the action research cycle, which included self-efficacy, attitudes, sense of belonging, and identity. In this section, I will define and summarize previous studies relating these factors to student success in STEM.

Self-efficacy is the perceived ability succeed at completing a particular task, which has been found to relate to academic motivation, interest, goals setting, effort, and persistence [152]. Self-efficacy is a well-known predictor of academic performance in STEM, including in predicting GPA [153]. Low self-efficacy has been shown to induce negative emotional responses, including anxiety [145], [152]. Student background characteristics, including gender, membership in groups underrepresented in STEM, and socio-economic class, have been shown to impact STEM self-efficacy [154], [155]. Various educational interventions have been shown to increase self-efficacy in STEM, which effectively give students the opportunity to practice professional STEM skills or interact with experts, including undergraduate research programs, problem-based learning, maker and design experiences, and effective mentoring [154]–[158]. Attitudes describe students' emotion, cognition, and intention towards a field [159]. Attitudes towards STEM are influenced by personal interest, self-efficacy, and perceived usefulness or applicability to their lives or values [159]. Positive attitudes towards a field have been related to achievement [160], [161]. Gender has been shown to have an impact on attitudes towards STEM, especially in traditionally masculine dominated fields like computer programming [159], [161].

Sense of belonging describes a student's feelings of connectedness and importance within an institution [162]. Students navigate their sense of belonging based on their environment and context, for example at the college, dorm, class, or field levels [149], [162]. For academic fields, including STEM, a critical component of belonging is congruence between personal beliefs and field values [149]. High sense of belonging in higher education is associated with academic success, emotional well-being, motivation, engagement, and retention, while lower sense of belonging is associated with lower emotional well-being [149], [162], [163]. Previous studies have shown that multiple identity-based factors, including gender, queerness, underrepresented minority status, and first generation status, may impact a student's sense of belonging in STEM [148], [149], [162]. Identity development is the process by which students grow to associate themselves with people of a particular context, including to STEM fields, institutions, and to affinity groups [164]. For example, a student develops STEM identity as they begin to 'see themselves' as a STEM person. STEM identity development is contingent on gaining competencies, becoming well-versed in cultural standards, having continued interest, and being recognized by others [158], [164]–[166]. STEM identity development correlates to academic achievement, high attitudes, and persistence in the field [165], [166].

6.3 Student-centered learning

STEM fields are highly interdisciplinary with many subdisciplines that require graduates to apply many levels of scientific and mathematical knowledge to complex problems. Because application of knowledge is a critical component to STEM, undergraduate studies must prepare students beyond memorization of facts and concepts; students must organize and connect knowledge so that it is 'usable' during problem solving [167], [168]. Traditional lecture-based courses, like those described in the Motivation, are based in the instruction paradigm, which maintains that a professors' goal in a classroom is to deliver content to students [169]. Previous research has shown that the instruction paradigm is not effective for the development of higher order application of material [167], [169], [170]; students taught primarily with this method have demonstrated difficulty using previously learned knowledge in new contexts [167]. To better prepare students for complex problem solving, experts called for an adaptation of the learning paradigm, which asserts that a professors' goal in a classroom is to encourage student learning [169]. As opposed to the instruction paradigm, the learning paradigm involves students in the responsibility for their learning – it is not enough that the instructors provide knowledge, students must engage with knowledge to build an understanding [169].

Active learning approaches give students the opportunity to engage with material, which promotes the learning paradigm. Broadly, an active learning exercise is any activity that requires more than passive listening from students [171]. Previous studies have found that active learning increases student learning compared to traditional lecture-based courses [172]. Despite this, traditional lecture methods were found to be the most common use of class time in STEM courses in 2018 [173]. Implementation of active learning can vary from incorporating low time commitment activities (e.g., think-pair-share, clicker questions, minute papers) to completely redesigning courses (e.g., flipped classroom, service learning). In most forms, active learning activities give students the ability to practice the application of material in a low-stakes environment where they can get immediate feedback on their logical thinking processes [171]. Active learning activities can also help in the development and maintenance of social-psychological indicators discussed above, such as self-efficacy, positive attitudes, and STEM identity. Project- or problem-based learning is particularly helpful in STEM, as students are given the opportunity to practice authentic problem-solving, which has been shown to help build self-efficacy and STEM identity [167], [174], [175]. These methods are often paired with cooperative learning, which allows students to learn from peers and develop effective teamwork skills [167], [176], [177]. In addition to these benefits, active learning can help students gauge their understanding of a concept and determine the level of effort needed until mastery, which can increase the amount of control they exert in their education [167], [168]. The amount of control a student has in their education is another important social psychological factor for academic success [175].

6.4 Climate in STEM

Given the importance of social context on student success, it is critical to examine the climate for historically underrepresented groups in STEM. Women and people of color historically receive less STEM higher education degrees and hold fewer positions in the STEM workforce than appropriate based on the US census [178]–[180]. A leaky pipeline analogy has been commonly used to describe the leaving of groups currently underrepresented in STEM from the field; however, this has been criticized for prioritizing increasing STEM graduates versus improving the climate for students [181]. In this work, we use a critical approach for characterizing the climate in STEM for underrepresented groups, which attributes the oppression of identity-based groups to societal forces rather than intrinsic differences between individuals [16]. We pair this with a strengths-based approach, which highlights the assets that individuals use to navigate society, rather than a deficit approach, which supposes that marginalized individuals have deficits that need to be "fixed" to integrate into society [16]. Various factors of the STEM and US sociopolitical environment contribute to a chilly climate for underrepresented racial or ethnic groups, women, first generation students, students of low socioeconomic background, LGBTQIA+ students, and disabled students, which contribute to low sense of belonging and difficulty developing STEM identity, limiting the ability for marginalized students to succeed despite their strengths, interests, and skills in the field [148], [149], [162], [179], [180]. In this section, I will briefly discuss some of the key components of the STEM climate that work to marginalize students with identities that differ from the majority, regardless of specific identity, followed by a brief discussion of how these factors compound for students who hold multiple marginalized identities.

Before discussing specific aspects of STEM culture that lead to marginalization, it is important to acknowledge the aspects of US society that contribute to inequity for underrepresented groups long before their STEM education. For example, underrepresented racial and ethnic groups are less likely to have adequate preparedness in science and math, a large contributor to STEM academic achievement, because of inequitable historical and societal distributions of wealth and resources to non-majority groups [180]. Societal views of gender, math, and technology contribute to lower self-efficacy and interest in these fields by women starting in Middle School [182]. As mentioned in the motivation, there is a dominant narrative concerning those who succeed in STEM, which reduces the sense of belonging for those whose personal identities are not congruent with or whose culture values are not represented in it [183]. Because the STEM field has largely been dominated by white, cis-heterosexual, abled men, the traits and values associated with STEM identities are also those that are traditionally held by white, cis-heterosexual, abled men [184]–[188]. Stereotypes that persist concerning STEM success include being non-social, individualist, competitive, willing and able to work for long hours, and completely objective [141], [188], [189]. These stereotypes exclude students from cultural backgrounds that prioritize family and working towards collective benefits [190], value social and non-technical skills [187], [191], and have caregiver or family responsibilities [188]. Master and Meltzoff (2020) further defined how stereotypes of success and individual identities impact retention in STEM, which can be seen in Figure 6-2. In this model, interventions were defined to help individuals with underrepresented status persist, including broadening the definition of STEM workers; a growth mindset, which emphasizes gaining competencies through effort as opposed to intrinsic ability; and increasing sense of belonging [187].

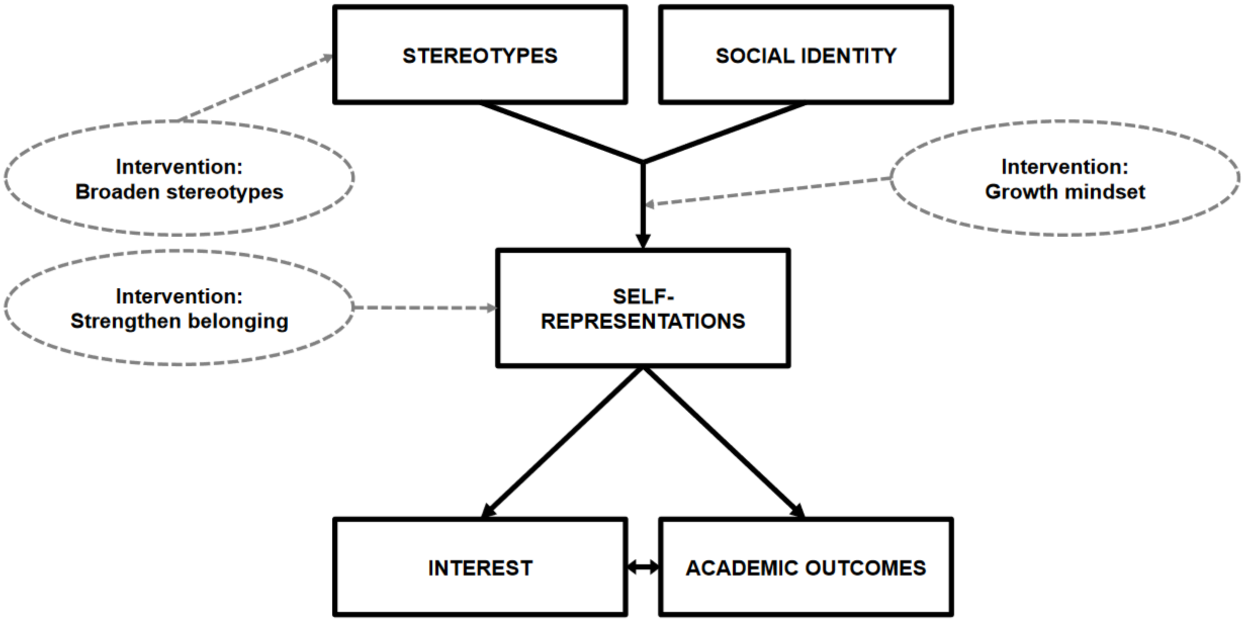


Figure 6-2. Impact of stereotypes associated with success in STEM and individual identities on STEM outcomes, from [187].

Other factors contribute to the chilly climate in STEM for individuals, including underrepresentation, isolation, stereotype threat, and microaggressions. Primary representation of individuals from the dominate identity groups further enforce the identity-based stereotypes of successful STEM workers [182], [192]. Furthermore, lack of role models with similar characteristics within peer and achievement groups leads to a difficulty socializing into STEM culture, developing STEM identity, and feeling a sense of belonging [141], [179], [186], [188]. Various factors contribute to feelings of isolation in STEM including social alienation by peers, absence of other individuals from their identity groups, and lack of acknowledgment of their identities, which leads to reduced sense of belonging in STEM and decreased emotional well-being [186]. The latter of these factors is particularly important as it contradicts the idea that a ‘colorblind’ view is beneficial to underrepresented racial groups. In fact, implementation of a colorblind framework for addressing diversity from healthcare administrators led to decreased engagement from racially diverse individuals compared to a multicultural framework [192]. While stereotypes exist regarding the traits of those who succeed in STEM, stereotypes are also imposed on underrepresented groups. In a chilly climate, individuals may fear their behavior or level of academic achievement may confirm negative stereotypes about their identity groups, i.e. stereotype threat, which is associated with lower motivation, academic achievement, and emotional well-being [187], [193]. Microaggressions are indicators of a lack of belonging or a negative view of identity groups often perpetrated by people with power [186]. Racial- and gender-based microaggressions are prevalent in STEM education and include undervaluing the achievements and STEM identities of individuals in these groups. Microaggressions contribute to the low sense of belonging and emotional distress of underrepresented groups in STEM [186].

While we use a critical lens to identify aspects of STEM culture that contribute to oppression of underrepresented students in general, it is important to acknowledge that these factors compound for individuals who hold multiple marginalized identities [186]. For example, the intersections of being non-male and non-white compound for Black women, causing cumulative disadvantage, i.e., their identities will have larger impacts than either Black men or white women [188]. Women of color are extremely underrepresented in STEM and have been shown to experience isolation, microaggressions, tokenism, and harassment [143], [186], [188], [189], [192]. Various interventions have been developed to improve the culture in STEM for underrepresented individuals with varying degrees of success, which often aim to increase sense of belonging or interest towards STEM materials [190]. However, further work should focus on dismantling the aspects of STEM culture that create a chilly climate and contribute to oppression of non-majority individuals.

6.5 Educational Data Collection Strategies

Experimental design to determine the impact of educational interventions should be primarily informed by the goals of the research project, i.e., whether the researcher is looking to determine if the intervention is better than traditional methods or if it is simply beneficial to students. When attempting to determine if an intervention is better than traditional methods, it is important to include a sufficient randomly selected control group and keep factors not related to the intervention consistent, like time of class, demographics of students, instructor, etc. If determining the benefits of an intervention without comparison to accepted methods, a time-based assessment without a carefully constructed control group may be sufficient [194]. For action research, the primary goal is to improve the developed intervention and gaining information about the widespread effectiveness of the information is secondary, so this work focused on characterizing time-based changes in metrics of individual success. The indicators of success that are assessed should be linked to the study objective, goals of the intervention, and theoretical framework. In this work, we applied social-psychological theory, so we used the indicators discussed in Section 6.2: attitudes, identity, and sense of belonging.

Researchers also need to design their data collection strategies based on the goals of their study, and these can include quantitative, qualitative, and mixed method approaches [4], [194]. Quantitative researchers rely on numerical metrics, like GPAs, grades on course assessments, or responses to Likert-type survey questions, to describe a population. An important assumption of quantitative researchers is the properties of these metrics can be used to gather knowledge about the characteristics of the population [3]. For quantitative research, the experiment should be constructed to ensure generalizability to the entire population by random sampling and large enough sample sizes, validity of measurements to the actual metric of interest, and reliability of measurement strategies over time [194]. Qualitative research relies on in depth data collection from an individual or, usually small, groups of participants with forms such as interviews, open-response survey questions, focus groups, ethnographies, etc. [4], [194]. As opposed to characterizing an entire population, qualitative researchers can gather information about individual experiences and the strategies an individual uses to navigate within the population [195]. Furthermore, qualitative methods are particularly helpful for exploratory studies, since it is easier to identify unexpected insights [194]. Many engineers are more comfortable with quantitative research because it is more similar to traditional engineering methods and is viewed as more objective than qualitative. However, careful experimental design, thorough description of experimental context, and thick description of the results can ensure that qualitative studies are credible, transferable, and dependable [194]. Researcher positionality is important for both quantitative and qualitative research, although examination and explicit statement of positionality is especially important for qualitative research as researchers naturally analyze information through the lens of their experiences and identities [3]. Mixed methods combine qualitative and quantitative strategies at various levels, which should be informed by the goals of the study [194]. In this work, we used mixed methods approaches to assess the effectiveness of interventions.

6.6 Personal Motivation

Chapter 7 Instructional Practices for Teaching Computer Programming: Applying Research-based Teaching Strategies

This chapter has been adapted from a peer reviewed version of this work:

R Rosario*, SE Hopper*, A Huang-Saad. “Applying research-based teaching strategies in a biomedical engineering programming course: Computer-aided diagnosis,” *J Biomed Eng Educ*, vol *2,* no 1, pp 41-55, 2022, doi:10.1007/s43683-021-00057-w.

* Authors contributed equally to this work.

7.1 Introduction

Computer programming is fundamental for the academic and professional success of biomedical engineers. Many undergraduate biomedical engineering (BME) programs across the United States have a computing component [196]. Additionally, a recent survey of BME faculty identified programming as the second most important skill for the future careers of biomedical engineers, just behind statistics and more important than design, regulatory materials, biomaterials, and system processing [197]. Thus, developing strong programming fundamentals is critical to the success of BME undergraduates. One important factor that impacts students’ success with computer programming is their attitudes towards the field [160], [161], [198], [199]. As mentioned in chapter 6, student attitudes are dependent on their perceived meaningfulness, interest, and self-efficacy with computer programming [160]. Furthermore, student identities can impact their attitudes towards programming; previous studies have found that women are less likely to have high attitudes towards programming than men [161], [200] Anecdotally, a large proportion of undergraduate BME students often have low attitudes towards, confidence in, and aptitude with computer programming, but this has not been investigated in depth previously [201].

Incorporating research-based instructional strategies can improve teaching techniques for computer programming. While engineering education researchers seek to make strategies generalizable for most classroom environments, strategies utilized by an instructor should be grounded in their learning objectives, classroom environment, and student population. Instructors can use practical action research to iterate on their implementation of educational strategies to optimize for their specific course. In this work, we describe an example of one action research cycle for improving classroom strategies in a computer programming module for biomedical engineering students, which can be seen in Figure 7-1. In this chapter, you will find an in depth discussion of all steps of the action research cycle, including a summary of relevant literature, implementation of research-based strategies, and assessment. Future recommendations for future iterations of this course can be found in Chapter 9. Specifically, we designed a 1-credit, 4-week applied programming course about computer-aided diagnosis (CAD) that implemented project-based learning (PBL) and scaffolding through lectures with active learning activities, labs, and an open-ended project. Social-psychological theory was used to assess the impacts of this combined intervention through quantitative analysis of conceptual knowledge and attitudes towards computer programming and qualitative analysis of open-response survey questions pertaining to the resistance towards the interventions.

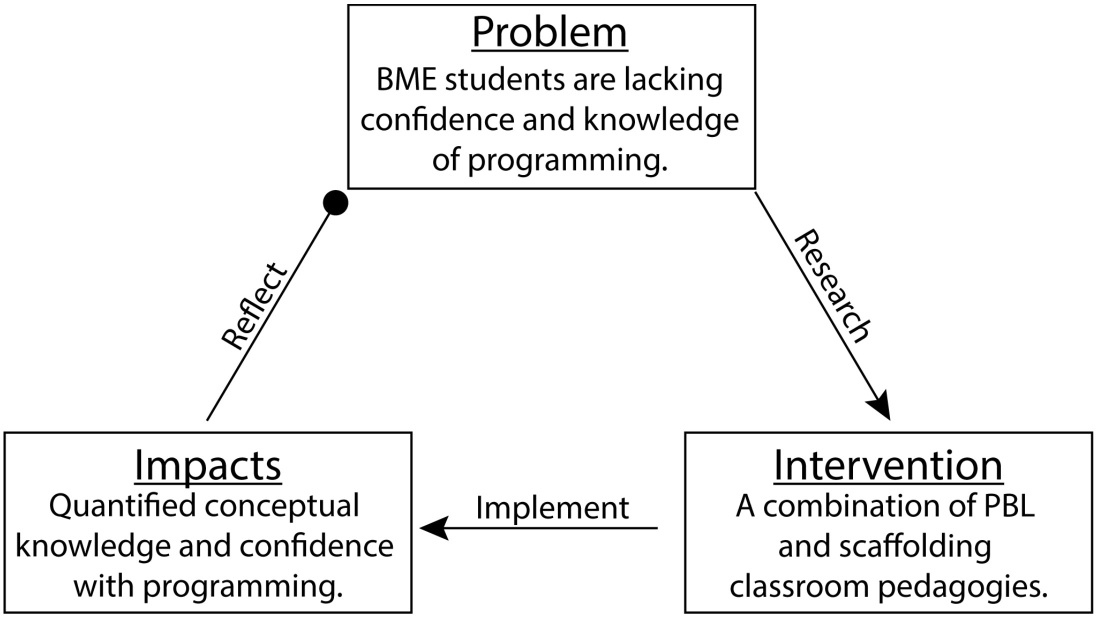


Figure 7-1. Action research cycle for improving teaching practices for computer programming to BME students.

7.2 Literature

7.2.1 Project-Based Learning

Project-based learning (PBL) is a common student-centered learning practice that has been used in engineering education, specifically to promote student engagement with design and increase professional skills [202]–[204]. In this method, students apply their knowledge to an open-ended, authentic problem in teams, which is meant be similar to the professional engineering experience [202], [203], [205]. Capraro and Slough defined PBL as "an ill-defined task within a well-defined outcome situated with a contextually rich task requiring students to solve several problems which when considered in their entirety showcase student mastery […]" [206]. Critical components of PBL include: well-defined outcomes, ill-defined tasks to promote self-directed learning, students working in cooperative groups to complete the task, instructors acting as a facilitator rather than provide explicit instruction, and projects having real-life applications [206]. PBL also often includes an end product as a summative assessment, often in the form of a device, program, design, report, and/or presentation [202], [207]. PBL is known to increase student's motivation, satisfaction with their work and learning, long-term knowledge, professional skills, self-directed learning skills, and engagement [208].

The implementation of PBL has been discussed and assessed in undergraduate engineering education. For example, at Massey University, the engineering curriculum has been redesigned with a focus on PBL in an effort to develop key professional competencies (knowledge acquisition, communication, problem definition, teamwork, system thinking, decision making, professionalism, and design process) in students [205]. Within PBL courses, students follow the project stages of comprehension of problem, creation of solution, critique, and communication. The benefits of PBL from this program include an increased learning of design principles, application of theory to practical problems, deep learning, and decreased rates of plagiarism. Students also had high satisfaction with PBL and its effects on their learning [205]. Furthermore, through a PBL-based civil engineering capstone class, Gavin suggests that PBL increased student confidence with group work, time management, and technical skills [209]. Students also had high satisfaction with PBL and high perceptions of their learning in a PBL course compared to lecture-based courses [209].

Instructor support is an important factor to consider when implementing PBL [202], [203], [205]. Scaffolding, described below, is one method that can be used in PBL classes to structure instructor facilitation. Pleiss, Perry, and Zastavker found poor student outcomes (low self-efficacy, negative view of instructor support, and poor motivation) in a PBL-based course that did not implement scaffolding compared to a PBL-based course that implemented scaffolding [156].

7.2.2 Scaffolding

The term “scaffolding,” in the context of education, was first described by Wood *et al.* in 1976 [210] and generally associated with the socio-cultural work by Vygostky [211]. In Wood *et al.*’s work, scaffolding was described as a process tutors use to support students in solving problems that is beyond the student’s individual ability [211]. While scaffolding is generally accepted as an effective teaching practice, scaffolding strategies are generally ill-defined [211]. van de Pol, Volman, and Beishuizen’s 2010 literature review sought to leverage education research to describe how teachers scaffold student learning experiences in the classroom and to rigorously define the process [211]. Their synthesis of 66 articles identified three characteristics of scaffolding in the classroom: (1) contingency, (2) fading, and (3) transfer of responsibility. Contingency is the adaptation of support to the student, which must be responsive and tailored to student needs. This requires the instructor to determine a student’s current competence before providing appropriate support. Fading is the gradual decrease of support. To be fading, the level of support must gradually decrease over time. This is closely linked to the final component, transfer of responsibility. To have a transfer of responsibility, the student must gradually take ownership over their learning.

Education research in scaffolding is largely based in the K-12 context. In one of the few studies addressing the effects of scaffolding on undergraduate engineering education, Mayer, Moeller, Kaliwata, Zweber, Stone, and Frank found that scaffolding single problem-based learning class sessions increased student performance on short-answer concept questions [212]. In this study, Mayer *et al*. operationalized scaffolding through ten-minute lectures on key concepts and guided handouts. The lecture and handouts introduced key concepts and guided students through the calculations necessary to solve the problem presented in the course. When compared to students in a problem-based learning session without scaffolding, the students in the scaffolded problem-based learning session scored higher on a post-course, short-answer concept test [212].

7.2.3 Implications for Design and Evaluation

In this section, we designed a four-week, one-credit module on introductory computer programming for BME students. To motivate students and promote interest in the topic, the course focused on an application of computer programming for the medical field. The module was a product of the U-M Biomedical Engineering Incubator [213]. Because students had no prior image processing experience, this module was designed to focus on both the acquisition and application of knowledge. We used traditional lectures, to promote gains in conceptual knowledge, particularly in the short-term [214], and PBL to mimic engineering professional realities by having students engage in the self-directed application of knowledge [203]. To strengthen the role of instructors as facilitators while implementing PBL and increase perceptions of instructor support and self-efficacy, scaffolding was incorporated throughout the module [156]. We defined four design goals for the module:

1. Students should demonstrate gains in programming skills as applied to the content.
2. Students should be able to identify clear applications for skills and knowledge from the module.
3. Students should demonstrate gains in conceptual knowledge.
4. Students should have positive attitudes toward the material and instructor support.

7.3 Implementation

7.3.1 Overview

Introduction to Computer-Aided Diagnoses (IntroCAD) was a one-credit, undergraduate module that met for three-hours, twice per week for four weeks in the winter semester of 2020. The module was designed by three graduate students, the first two authors and a classmate, enrolled in the University of Michigan BME Instructional Incubator in Fall 2019. The first two authors (the graduate student teaching team) elected to co-teach the module in winter 2020 with the third author as their faculty mentor. The goal of the module was to provide students with a basic knowledge of digital image processing in the context of medical applications and to increase students' skills in image processing, computer programming, teamwork, and communication, as characterized by the learning outcomes in Table 7-1. There were three fundamental components: 1) lectures with active learning exercises, 2) labs, and 3.) a final project (Figure 7-2). Lectures and labs were designed to introduce foundational coding and image processing skills and concepts. The final project, a PBL exercise, allowed students to apply that knowledge to an authentic problem.

Both formative and summative assessment were used to provide feedback, gauge student perceptions, evaluate growth in conceptual knowledge and skills, and assess completion of learning outcomes (Table 7-1). Formative assessments included responses to lab questions, in-class activities, three surveys, and daily muddiest points [215], which accounted for 30% of the total grade. For each lab, students were asked to submit scripts and answer 5-6 questions, which is described in more detail in the lab section below. Lab responses were designed to address learning outcomes 1, 2, and 4 (Table 7-1). Students were graded on activities from lectures, which were designed to check for understanding of the content that was covered in class. The surveys helped the graduate student teaching team assess completion of design goals and adjust class session plans based on students' expectations, areas of confusion and interest, and general feedback. Students were asked to submit a muddiest point for every class session, which asked students to identify the most confusing part of the class which instructors would address at the beginning of the next class [215]. These muddiest points and surveys helped the graduate student teaching team to adjust class plans to address areas of greatest confusion, enabling the contingency required when implementing scaffolding. Summative assessment from the final project accounted for the remaining 70% of the total grade, consisting of a script (30%), final report (20%), and presentation (20%). The script assessed learning outcomes 1, 2, and 3, while the report and presentation assessed outcomes 4 and 5. Due to unexpected impact of COVID-19, the last class (Week 4, Thursday) was cancelled.

Thirteen students enrolled in the module (Table 7-2). Although the module was initially designed for sophomore BME students, 8 out of 13 students had already completed their sophomore year. In a pre-module survey, all students expressed that they had at least some prior introductory-level coding experience and had previously used MATLAB.

Table 7-1. Learning outcomes and the corresponding module element designed to meet that outcome.

Number	Learning Outcomes	Portion of module covered in
1	To apply automated image processing techniques to medical images	Lecture, labs, final project
2	To implement industry best practices to create organized, efficient, and understandable code	Lecture, labs, final project
3	To work as a team to design an algorithm to identify and describe illness or injury	Final project
4	To critically evaluate methods used in scripts	Labs, final project
5	To communicate the motivation for creating their scripts, methods, results, and broader implications and future extensions of their final scripts.	Final project

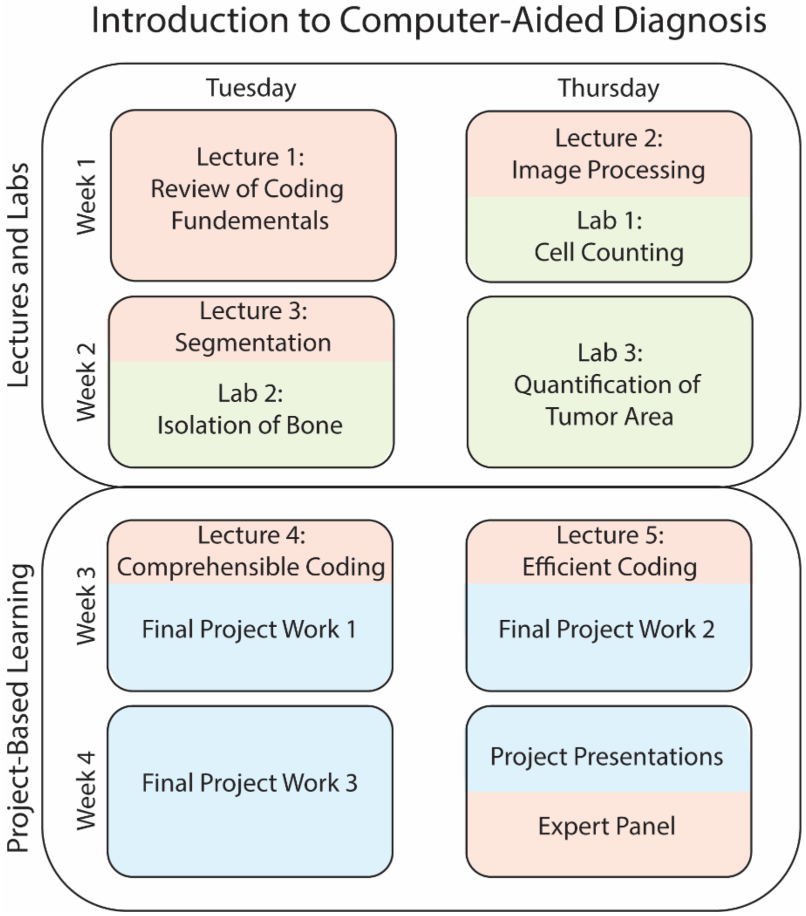


Figure 7-2. Schematic of module schedule with lectures (orange), labs (green), and final project (blue) indicated.

Table 7-2. Student demographics for those enrolled in IntroCAD (N=13)

Class level: 1st year: 0 2nd year: 5 3rd year: 4 4th year and higher: 4
Gender: Male: 5 Female: 8
Formal programming experience: None: 1 Only introductory courses: 6 Higher level programing courses: 6
Confidence with Image processing: Strongly agree: 2 Somewhat agree: 1 Neither agree nor disagree: 3 Somewhat disagree: 5 Strongly disagree: 2

7.3.2 Lectures

Over the eight class sessions, five lectures (Figure 1) were presented. Lectures 1, 4, and 5 focused on general coding skills, supplementing the university's introductory coding course for engineers. Lectures 2 and 3 introduced concepts relevant to image processing. All lectures except the first lasted 30 minutes or less. Lectures incorporated active learning exercises and facilitated discussions. Each class started with a clear explanation of the daily learning objectives and ended with a muddiest point exercise [215].

Lectures 1-5 were scaffolded; lecture content was informed by student survey responses and designed to address lab questions and muddiest points, demonstrating contingency. Lectures 1 and 2 included more thorough descriptions of concepts, and lectures 3-5 evolved to be more open-ended and included student-led discussions about problems in the field and issues students were facing in their final project. This progression illustrates fading and transfer of responsibility, two critical components of scaffolding.

Lectures 1, 4, and 5 heavily incorporated active learning exercises. Lecture content was presented to students in 3-10 minute intervals, which were immediately followed by sample problems. Students completed the sample problems, asking their peers for help as needed. For lecture 1, which reviewed coding fundamentals, a phone-based application was used to poll student responses to concept-based questions throughout the lecture. If most students answered incorrectly, then additional instruction was provided, thus demonstrating contingency. For lectures 4 and 5, which covered coding for comprehensibility and efficiency, the graduate student teaching team led a discussion where students discussed their evaluations of sample scripts in terms of the criteria for comprehensibility and efficiency that were reviewed in lecture. To incorporate active learning in all lectures, students were often asked to consider questions individually before discussing in small groups and sharing to the class (think-pair-share), think of real-world applications, critique methods, and summarize key concepts from labs and lectures [171].

7.3.3 Labs

Labs were self-paced image processing exercises that students completed individually. There were three labs in total, which introduced loading image data, segmenting images, and quantifying features (Figure 7-2). As an example, the workflow from the third lab is shown in Figure 7-3. Each lab was designed to meet the following five criteria: (1) a focus on a real-world problem, (2) a beginner-level set of instructions and questions to address the problem, (3) advanced-level open-ended extra credit questions to address a nuance on the problem, (4) an accumulative build-up of knowledge with progressively less detailed instructions, and (5) a low-stakes checklist for formative assessment.

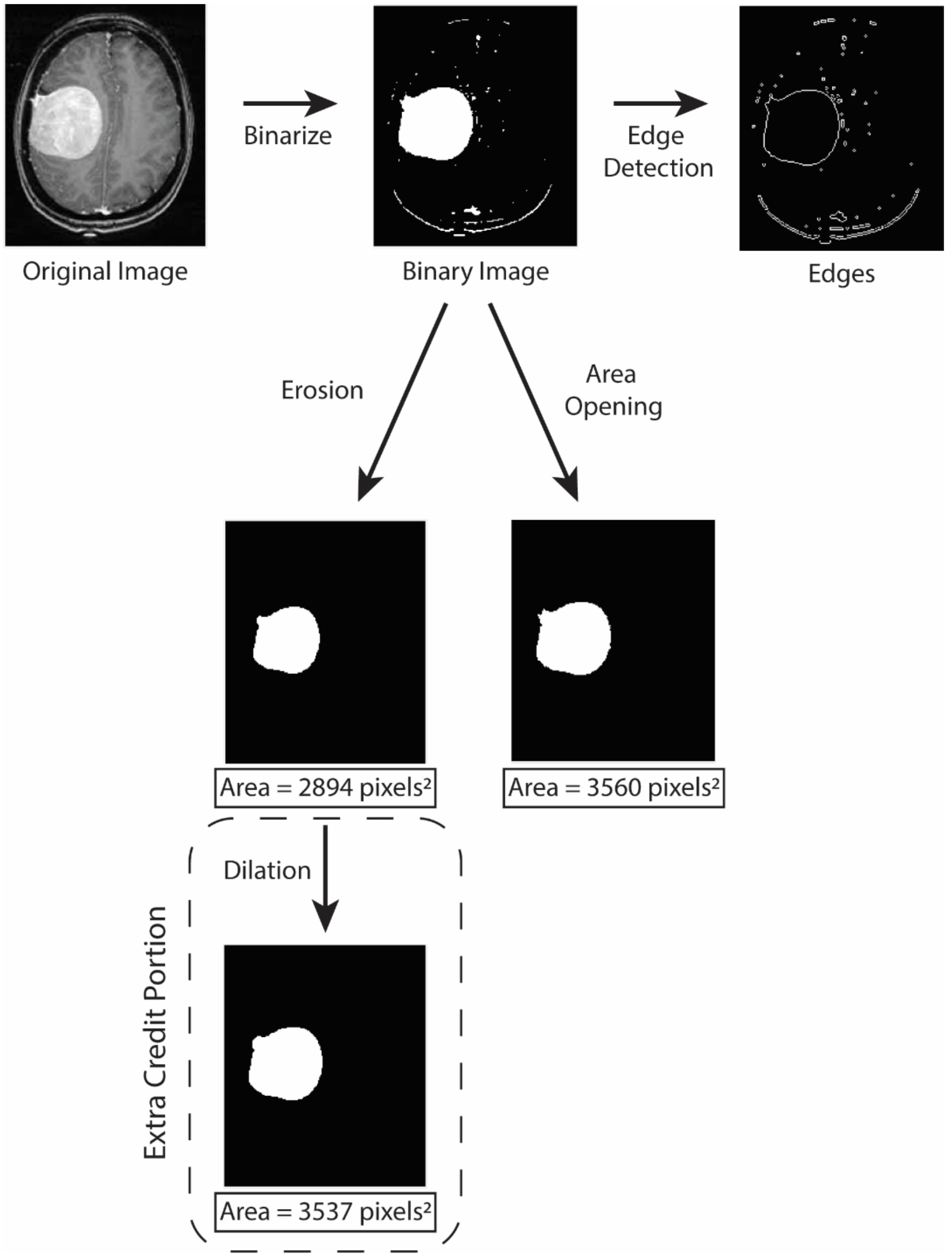


Figure 7-3. Workflow of Lab 3: Quantifying Tumor Area. Students were given the original image and needed to conduct sequential code-based image processing steps to isolate and quantify the area of a brain tumor using two different methods. Students then answered questions related to the validity of both methods.

Real-World Problem. For each lab, students were tasked with solving an authentic task associated with medical images, which has been shown to increase engagement with material and learning [216]. This allowed for unexpected errors that required students to engage in basic inquiry to correct. These errors occur because real-world data has inherent noise and deficiencies that can make pre-set tasks difficult. Finally, using real medical images prepared students for their final projects, where students applied concepts from labs to a larger, unstructured real-world problem.

Beginner-Level Instructions and Questions. Each lab included beginner-level instructions and questions that students with minimal coding experience should have been able to complete. These instructions and questions were designed for the student who can write a simple script using mathematical operations, variables, conditionals, loops, and function calls; all skills that are learned in the first-year coding course for engineers at the institution. Student confidence with these skills was also assessed using the pre-module survey, demonstrating the scaffolding component of contingency. Using only that introductory knowledge and instructions included in handouts, students were expected to be able to complete the exercises. In some cases, this was accomplished by providing several lines of code that students could directly insert into their scripts. When code was provided to students, a corresponding follow-up question that asked students to explain the function was included to confirm comprehension. Lab instructions and questions were reviewed by four BME faculty, one Ph.D. student in engineering education, and one postdoctoral fellow in engineering education for clarity prior to the launch of the module.

Expert-Level Extra Credit Questions. Recognizing that students of varying levels may enroll in the module, complex, open-ended extra credit questions that required more advanced knowledge and inquiry to solve in the lab handouts were also included. These questions were designed for students with more experience in computer programming and covered concepts that were not explicitly introduced in lecture. The extra credit questions took two forms, either asking a student to explain a concept that was introduced but not explored in the lab activity or implementing a new piece of code that necessitated the use of functions not discussed in the module. To complete the extra credit assignment, students often needed to search MATLAB documentation to understand the nuances of functions or to identify a function that would meet their needs. The more advanced extra credit questions required outlining and iteration to complete. By including questions that were targeted at both beginner- and advanced-level students, the lab activities incorporated contingency.

Progressively Less Detailed Instructions. Fading was explicitly incorporated in the design of the labs. Each successive lab activity required the use of skills and knowledge that were introduced in prior lab activities with the goal of introducing students to a range of skills and serve as a starting point for their final projects. This was accomplished in the lab handouts through careful wording of the instructions. Lab handouts included more detailed instructions when first introducing a skill or concept with less detail in subsequent labs. For example, in lab 1, one step had students convert an image to grayscale: "Since this is an RGB image, we need to convert to grayscale before we can binarize or perform our other operations. Use the function rgb2gray to create a new image matrix." Much less detail was provided for this step in Lab 2, where a similar step instructed students to "convert the image to grayscale," with no additional instruction. If students were unsure how to perform any of these steps, they were referred to prior lab materials or the MATLAB help directory. The lab handouts demonstrated two of the critical components of scaffolding: fading and transfer of responsibility. Table 7-3 provides specific examples for how each lab incorporated these first four lab design criteria.

Formative Assessment. Lastly, lab handouts provided a low-stakes opportunity for students to identify their current knowledge level and gaps that needed to be addressed. Incorrect responses on the lab handout resulted in a small (1-2%) point reduction. Full credit was awarded if the script compiled, all steps of the lab were followed, all questions were answered in 1-2 sentences, and all figures were created with descriptive labels. Lab assignments were graded and returned before the due date of the next lab, which allowed students to address their issues in the next lab. The labs provided beginner-level students with a low-stakes opportunity to acquire the skills necessary to effectively contribute to the final projects.

Additional Considerations. While not a key component of the design process, it should be noted that students were asked to sit with their project teams when completing the labs to encourage peer-to-peer learning [177]. When a student had a question about the lab, the graduate student teaching team encouraged the students to first discuss the question with their project teams. If the team was unable to answer their question through discussion, instructors would re-enter the discussion. By the end of the third lab, most student questions were answered by their peers without instructor guidance.

Table 7-3. Description of how four of the lab core components were included in each activity.

Component	Lab 1	Lab 2	Lab 3
Real-world problem	Count cells from fluorescent microscope images	Segment bones from knee x-ray images	Quantify tumor size from brain MRI scans
Beginner-level instructions and questions (contingency)	Count three isolated cells from a high-contrast image with step-by-step, highly detailed instructions. Requires explanation of provided code with minimal independent implementation. **Learning objectives:** • Define image properties • Manipulate images using arithmetic operations and built-in functions	Isolate bones from x-rays. Uses images with less contrast between the region-of-interest and the background. Requires some independent code implementation. **Learning objectives:** • Define and implement image morphological operations • Identify issues caused by morphological operations	Isolate and quantify tumor size from MRI scans with very low contrast between the region-of-interest and background. Requires nearly independent code implementation. **Learning objectives:** • Identify image processing difficulties caused by low contrast • Interpret MATLAB help
Advanced extra-credit (contingency)	Count cells from an image with many highly clustered cells. Requires logic and/or functions not used in the beginner lab.	Redo the lab using built-in functions that were not introduced in the lab instructions.	Create a metric and implement a script to identify whether the tumor is likely to be malignant based on its shape.
Example of progressively less detailed instructions (fading)	Explicit instructions are provided for grayscale conversion and binarization: *Since this is an rgb image, we need to convert to grayscale before we can binarize or perform our other operations. Use the function rgb2gray to create a new image matrix.* *Use the imbinarize function to binarize the image.*	Explicit instructions are provided only for binarization because a more complex process is used: *Convert the image to grayscale* *We will create a binary version of the image with a threshold value calculated by the ‘ sobel’ operator. Use this threshold value with the edge function to create a binary image. Use the following code to do so:* *[~, threshold] = edge(<grayscale image>*, 'sobel');	Because (1) grayscale conversion and binarization were previously used and (2) binarization requires a grayscale image, only the final instruction was provided: *Binarize the image.*

7.3.4 Final Project

The last half of the module was devoted to the final project, where students extended what they learned in the first four sessions and incorporated knowledge from other biomedical engineering domains. Pre-module survey results were used to create groups with evenly distributed coding ability. Groups were tasked with selecting one of eight problems. Each problem instructed students to use image processing to quantify a clinical parameter, such as “quantify the age and size of a fetus.” Students were also responsible for finding their own radiographic images from medical databases. None of the problems were previously used as an example in the module. Each problem provided a well-defined task with clear real-life applications.

To solve the problem, students needed to seek out new knowledge. Specifically, they needed to understand the clinical problem, to understand the corresponding physiology, and gain additional imagine processing skills. As a result, the graduate student teaching team observed the students engage in self-directed learning. For example, one student group chose to analyze a computed tomography (CT) scan to determine whether a patient had kidney stones (Figure 7-4). The students were unfamiliar with the causes of kidney stones, their appearance in medical images, and relevant clinical markers when making a diagnosis. To address this knowledge gap, the students looked to general online sources and academic papers on kidney stones. After sharing knowledge among group members, the students applied this conceptual knowledge of the pathology to their iterative algorithm development, where they suggested potential identification methods, acquired missing imaging processing skills-based knowledge, implemented their identification method in MATLAB, and evaluated the algorithm’s performance. The students’ process was emblematic of cooperative, self-directed learning that occurs during PBL when the project is ill-defined [206].

Consistent with the definition of PBL by Capraro and Slough, the module instructors facilitated the learning process and modeled reasoning strategies rather than provide explicit instruction during the final project portion of the module [206]. In their role as facilitators, the instructors asked questions and pointed students toward relevant resources rather than directly answering their questions. Again, looking at the kidney stone group, the students wanted to create a more versatile script by using an automated thresholding method rather than a hard-coded value as was done in the guided labs. Initially, the student group asked the instructors for guidance. Rather than provide an algorithm, the instructors prompted the students to brainstorm and evaluate potential automated thresholding methods. Ultimately, the students created their own algorithm that used an intensity histogram to choose an informed threshold value for their images. Through this self-directed learning process, the students discovered informed thresholding techniques that went beyond the skills covered in the first two weeks of the module. Without instructor facilitation, the students would have been unlikely to engage in self-directed learning and move beyond the materials covered in the initial lab sections.

In addition to facilitating group discussion and self-directed learning, the graduate student teaching team guided students through the PBL project phases used at Aalborg University to model effective project management skills, as seen in Table 7-4 [202]. Students were assigned a project planning worksheet, which prompted them to complete project phases 1-5 by the end of the second week of the module. In phases 1-3, students defined the problem by providing a brief background on the body system and/or pathology and by identifying what they plan to quantify in their chosen medical image. In phases 4-5, students began to solve the problem by identifying which image processing techniques they will likely need to use and by developing a pseudocode outline of their final script. Completing this worksheet prepared students for the final project work sessions, where they focused on implementing, iteratively improving, and evaluating the performance of their final scripts.

Table 7-4. Implementation of Project Phases framework from Aalborg University in IntroCAD [9].

Project Phases	Implementation	Portion of Module
1. Initiating the Problem	Description of problem statement and identification of pathologies	Project handout and planning sheet
2. Problem Analysis	Motivation of problem statement and introductions of report and presentation	Final report
3. Definition and formulation of problem	Description of problem statement	Project planning sheet
4. Problem solving methodologies	Lectures of image processing basics and implementation in labs; self-directed learning during project-based learning	Project planning sheet and final report
5. Demarcation	Discussions within project groups and guided question from instructors	Project work time
6. Solving the problem	Iterative development of scripts	Project work time
7. Implementation	Project scripts	Final script
8. Evaluation and reflection	Critical analysis within written report and presentation	Project work time and final report

At the end of the module, students were asked to turn in three assignments for their project: a script, report, and presentation. The final script demonstrated students' skills-based knowledge gains from the project. The final report provided students an opportunity to evaluate how well their script addressed the posed problem and to abstract the knowledge acquired during from the specific problem and toward more general problems of image processing. This reflection is a key characteristic of PBL defined by Kolmos and de Graaff [202]. The final presentation allowed students to share learnings from their project across student teams and with our expert panel, who could then model expert-level image processing thinking to the students. Because the week 4, Thursday class was cancelled, teams were not graded on oral presentations, but only on their digital presentations. Summative assessments are summarized in Table 7-5, along with the grading criteria.

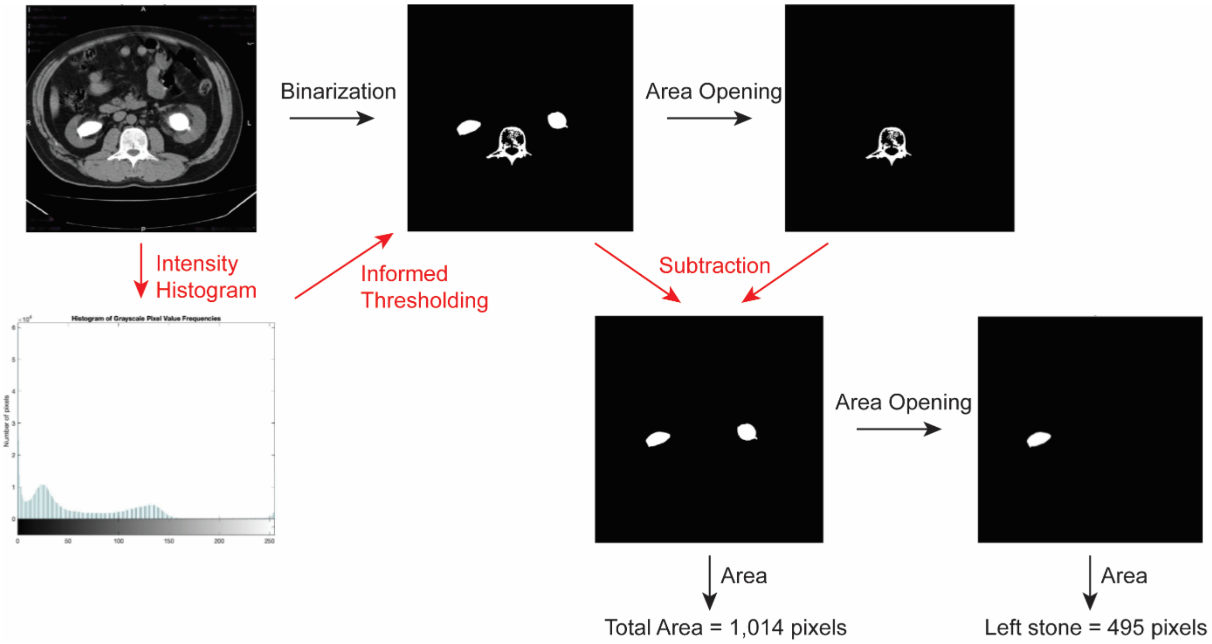


Figure 7-4. Representative final project submission. For this project, the team segmented and quantified cystic kidney stones. The team used thresholding based on average intensity values to isolate the kidney stones and spine from the original image, opening to isolate the spine, and then image subtraction to isolate the kidney stones. Image processing techniques not covered in class lectures or labs are shown in red.

Table 7-5. Deliverables for the team-based final project along with the learning outcome they fulfill, guidelines given to students, and grading criteria.

Final Project component	**Relation to learning outcomes**	**Deliverable**	**Grading Criteria**
Script	1, 2, 3	A MATLAB script that segments, quantifies, and creates figures relating to an injury or illness	Meets specifications, readability, documentation, and efficiency
Presentation	5	A 10-15-minute presentation on the background and motivation, methods, results, and discussion	Content, oral communication, and organization
Report	4, 5	A < 3-page report with background and motivation, methods, results, and discussion	Content and formatting

7.4 Evaluating Student Progression

Surveys and student-generated concept maps were used to document student progression throughout the module. Specifically, students' changes in perceived skills-based knowledge, perceived applicability of content, attitudes toward instructor support, and conceptual knowledge were measured. These modes of evaluation addressed the four goals that were established during module design. Data collection was approved by University of Michigan Internal Review Board (HUM00176990).

7.4.1 Survey Design, Collection, and Analysis

Survey questions were based on the previously validated scale for measuring attitude towards computer programing (AStCP) [161], [199] and adapted by Baser [161]. Survey items assessed usefulness of and confidence in learning computer programming (pre- and post-) and with image processing (pre-, mid-, and post-) with a 5-point Likert-type scale, which asked students to rate their agreement with statements with provided answers *strongly disagree, somewhat disagree, neither agree or disagree, somewhat agree,* and *strongly agree*. In addition to the Likert-type questions in the mid-module survey, students were also asked to describe what was and was not working well in the module. Similarly, post-module, students were asked about perceptions toward PBL, instructor support, and knowledge and confidence with computer programming and image processing. Students were asked to describe their expectations and whether those expectations were met. Each of the three surveys can be seen in Appendix A. Surveys were created and distributed using Qualtrics.

Twelve out of 13 students completed the survey at all three time points. Student responses were paired and analyzed with a non-parametric Wilcoxon Rank Sum test to measure the effect of IntroCAD on student attitudes toward the usefulness of and confidence in computer programming between the pre- and post-module. A non-parametric Wilcoxon Rank Sum test was used because of the small sample size. Population statistics rather than sample statistics were used for all tests because the analyses were designed to measure changes in attitudes for students in this module rather than a general population of biomedical engineering students.

7.4.2 Concept Maps

Concept maps were used to examine changes in conceptual knowledge over time. A concept map is a diagram where distinct concepts are connected by propositions. From a constructivist perspective, a concept map is a physical depiction of conceptual knowledge in the form of an interconnected web of facts, structures, and ideas [217], [218]. This diagrammatic method of organizing conceptual knowledge was formalized by Novak and Gowin, originally as a method of organizing clinical interview data [219]. Since their creation, the uses of concept maps have multiplied, and are now used as a method of assessing depth and breadth of conceptual knowledge, interconnectedness of concepts, and student misconceptions [220], [221]. The use of concept maps as an assessment method in engineering education has grown over the past two decades, providing insight beyond what is provided by traditional assessment methods into how students generate conceptual knowledge [222]–[227].

In this module, students generated concept maps during the first class session, mid-module, and post-module. At the first time point, the process for creating concept maps was reviewed and an example was discussed as a group. After the initial instructional session, students independently generated concept maps given the initial bubble “Image processing”. Examples of student-generated concept maps can be seen in Figure 7-7 and Figure 7-8. No time limit was given to generate concept maps, but most students completed their maps within 10-15 minutes.

Concept maps were scored using an adapted version of the validated rubric proposed by Besterfield-Sacre *et al.*, which assesses concept maps based on comprehensiveness, organization, and correctness (Table 7-6) [220]. Besterfield-Sacre *et al.* used this rubric to quantify discipline-specific conceptual knowledge growth of engineering students over time [220]. Comprehensiveness describes the breadth and depth of a concept map. In this context, we used comprehensiveness to assess whether students sufficiently included the module content. For this module, students were expected to include concepts related to image acquisition, image properties, fundamental coding skills, segmenting, morphological operations, and quantification for a medical diagnosis. Organization describes the physical layout of a concept map, based on hierarchy structure and interconnectedness of knowledge. A higher organization score indicates expert-level conceptual knowledge, highlighting the hierarchical nature and interconnectedness of concepts. Correctness measures the validity of concepts and links between concepts. Comprehensiveness and organization are measures of content coverage, where students are awarded points for including additional complexity. Correctness is a measure of validity, where points are taken away for incorrect usage of terms or links between concepts.

Table 7-6. Holistic scoring rubric adapted from Besterfield-Sacre et al. [220]

Criteria	**3**	**2**	**1**
Comprehensiveness— Covering content completely or broadly	The knowledge is very simple and/or limited. Minimal coverage of content. No extensions beyond what was covered in the module.	Some content is covered. There is one extension beyond what was covered in the module, but it is not fully developed.	Covers nearly all content and includes at least one fully developed extension (i.e., there is hierarchy level below that extension).
Organization— Arranging by systematic planning and united effort	Hierarchies have no cross-links between concepts and no branch structure.	There is at least one cross-link between concepts and at least one branching hierarchy.	There are multiple cross-links and branching hierarchies. Or uses a net-like structure with multiple feedback loops.
Correctness— Conforming to or agreeing with fact, logic, or known truth	The map in naïve and contains misconceptions about the subject area; inappropriate words or terms are used. The map documents an inaccurate understanding of some subject matter.	The map has some subject matter inaccuracies; most links are correct.	The map integrates concepts properly and reflects an accurate understanding of subject matter meaning with few or no misconceptions.

Prior to scoring the student-generated concept maps, the first two authors scored a set of six concept maps generated by doctoral students and faculty that use image processing techniques in their research. This was done to obtain consistency when scoring concept maps with the rubric. After the initial training, the instructors independently scored all student-generated concept maps, which were de-identified and scored in a random order. The first two authors then met to discuss scores and reach a consensus score for each concept map and criterion. A composite score was generated for each concept map by taking the sum of scores across rubric criteria.

To assess changes in conceptual knowledge as demonstrated by concept maps, the concept map composite score was examined over time. The median composite scores for module pre- to post-module were compared using a one-sided paired Wilcoxon rank sum test. Following quantitative analysis of concept map scores, qualitative document analysis of the concept maps was conducted for a select number of students. Two concept maps from two students that were representative of the changes in holistic scores observed from pre- to post-module were chosen for document analysis. One student had minimal growth in holistic score, while the other had substantial growth. Student in-class assignments and responses to survey questions were used to inform conclusions on changes in conceptual knowledge.

7.5 Findings

7.5.1 Surveys

Results of the Wilcoxon statistical analysis for student's attitudes toward the usefulness and confidence with computer programming are presented in Figure 7-5. Significant increases were found between pre- and post- time points for confidence with image processing and coding ($p < 0.05$). Mean responses for perception of increase in knowledge and confidence from IntroCAD can be seen in Table 7-7, along with the average mean score for the 29 questions relating to attitudes toward instructor support.

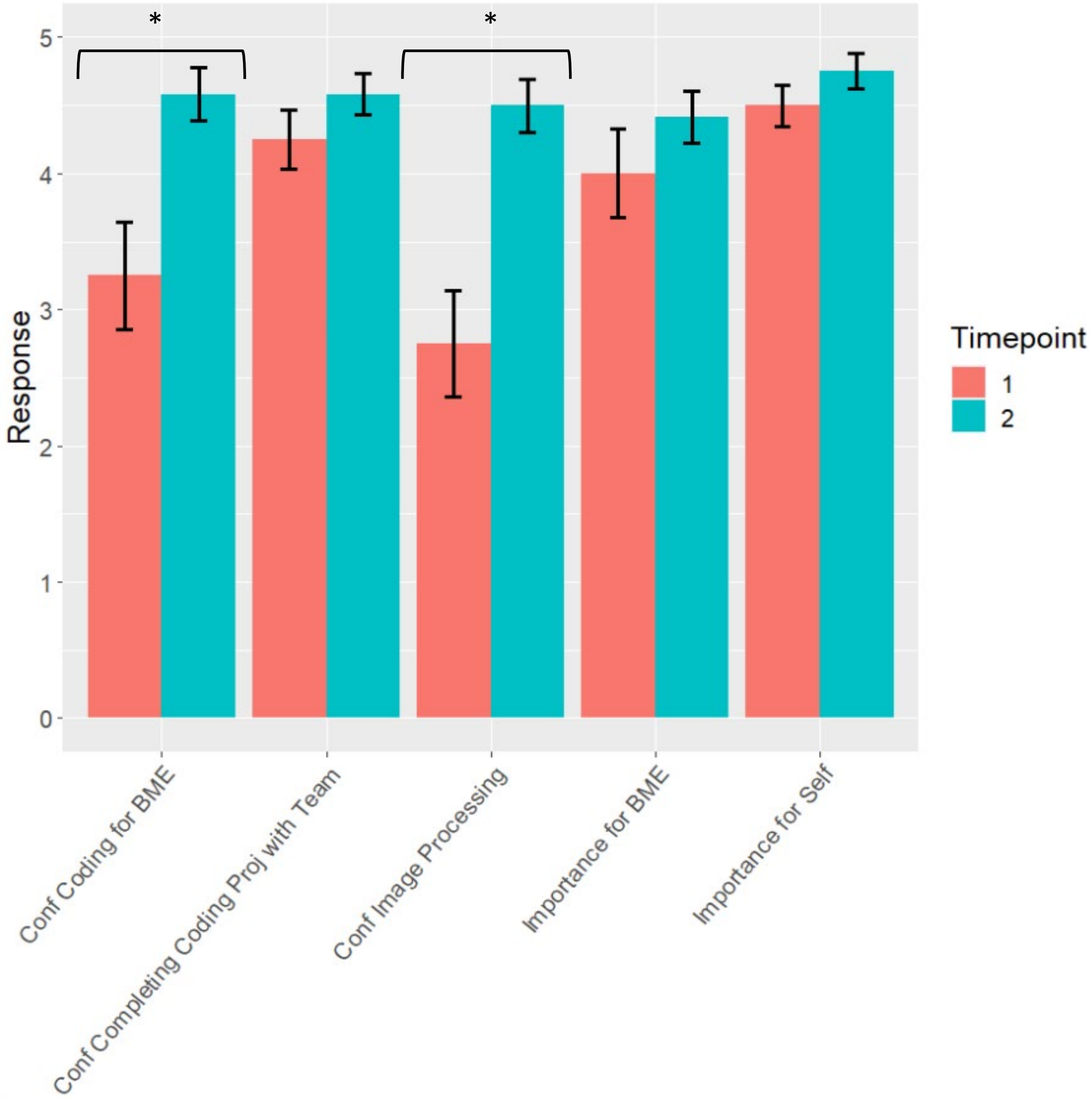


*Figure 7-5. Mean and standard deviation for responses from 5-point Likert-type scale questions concerning confidence and usefulness of computer programming and image processing at timepoints 1 and 2. Significant gains are indicated through *, where p<0.05.*

Table 7-7. Results of perceived increase in knowledge and confidence from Likert-scale questions out of 5 from the post-module survey. Mean +/- standard deviation and median are shown.

Question	**Mean**	**Median**
IntroCAD increased my knowledge of image processing	4.9 (± 0.28)	5
IntroCAD increased my knowledge of computer programming	4.7 (± 0.62)	5
IntroCAD increased my confidence in computer programming	4.6 (± 0.76)	5
The PBL techniques used in IntroCAD increased my learning	4.5 (± 0.76)	5
Average attitudes toward instructor support	4.5 (± 1.12)	5

In the mid-module survey, students identified three main areas that were working well: the utility of lectures and labs, helpfulness of in-class work time, and small group work. Students mentioned that labs and lectures, specifically "real-world examples" were useful and helpful for them, showing that they could see the applicability of the material. Regarding the module structure, one student responded, "the class format, which includes a 1-hour lecture and 2 hours of interactive lab time, gives a good balance of learning and practice of the material," while another said "I like the short lectures before the lab. Also, having help throughout the lab definitely helps me understand it more." Students had high perceptions of the proposed pedagogy at the mid-module timepoint. When asked what they would change about the module going forward, seven students said nothing, and the other responses identified two weaknesses of the module: the simplicity of content covered and rushed nature. Multiple responses said the module was not difficult enough or was too general, while other students said it moved too quickly through material.

In the post-module survey, 11 students said their expectations were met while one said they were "kind of" met. The reasons students gave for why their expectations were met included learning, practicing, and increasing confidence in coding and image processing. The student who did not have their expectations met, critiqued the module by saying "it was more of an image processing class disguised as BME related. Most of the material could have been self-taught with MATLAB." This is related to the identified weaknesses of the module from the mid-module survey. When asked what they would change about the module, five students did not have suggestions. The other students' suggestions fell into the following categories: increasing the difficulty, increasing the depth of material, and increasing the amount of critical thinking in the module. One response said, "make this [module] harder or go deeper, it's an interesting topic that we didn't get into much." Another response said, "making the labs more open-ended and having students think more critically about how to process the images."

7.5.2 Concept Maps

Results for student holistic scores can be seen in Figure 7-6. Nine students completed both the pre- and post-module concept map. Student concept map holistic scores steadily increased throughout the semester. There was a statistically significant increase in median concept map score from pre- to post-module (p = 0.021).

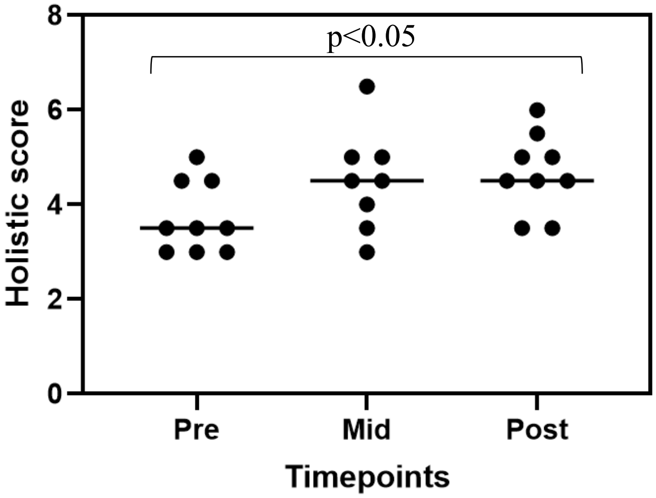


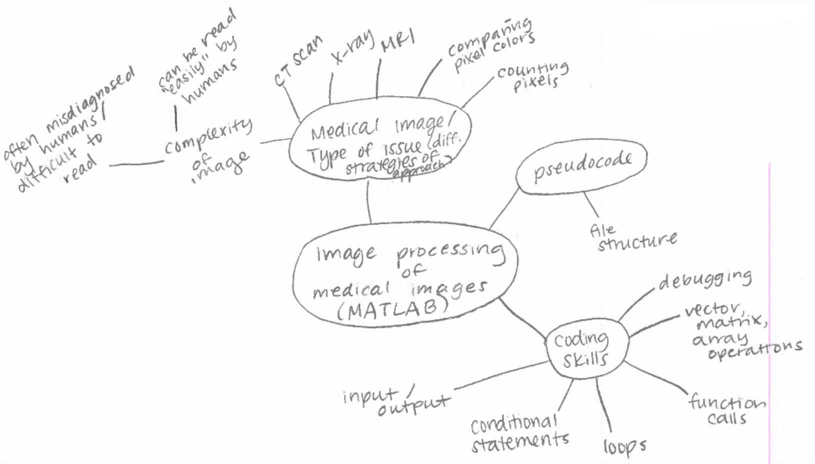
Figure 7-6. Concept map scores for all students that completed the assessment pre-, mid-, or post-module. A significant increase was found between pre- and post-module.

7.5.3 Document Analysis

Student concept maps were analyzed to better understand what differentiated a student with high pre-to-post gains in concept map holistic score from a student with low gains. One student from each group was chosen. The maps were then examined for how connections between concepts evolved and how that evolution related to student survey data.

Student 1: Alicia. Alicia demonstrated high growth in concept map holistic score from pre- to post-module (Figure 7-7). Alicia was a second-year woman in the BME department. She took AP Computer Science in high school and an introductory engineering programming course prior to enrolling, and she was co-enrolled in a 200-level programming course taught through the university's computer science department that focused on data structures and did not cover image processing, which gave her more coding experience than her peers in IntroCAD.

Student 820 Pre-Course



Mid-Course

Post-Course

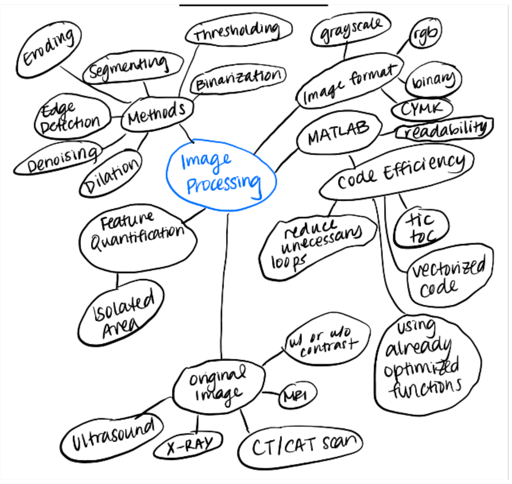


Figure 7-7. Concept maps from Alicia, who demonstrated high growth in concept map holistic score pre- to post-module.

Alicia demonstrated high growth in concept map holistic score from pre- to post-module (Figure 7-7). Alicia was a second-year woman in the BME department. She took AP Computer Science in high school and an introductory engineering programming course prior to enrolling, and she was co-enrolled in a 200-level programming course taught through the university's computer science department that focused on data structures and did not cover image processing, which gave her more coding experience than her peers in IntroCAD.

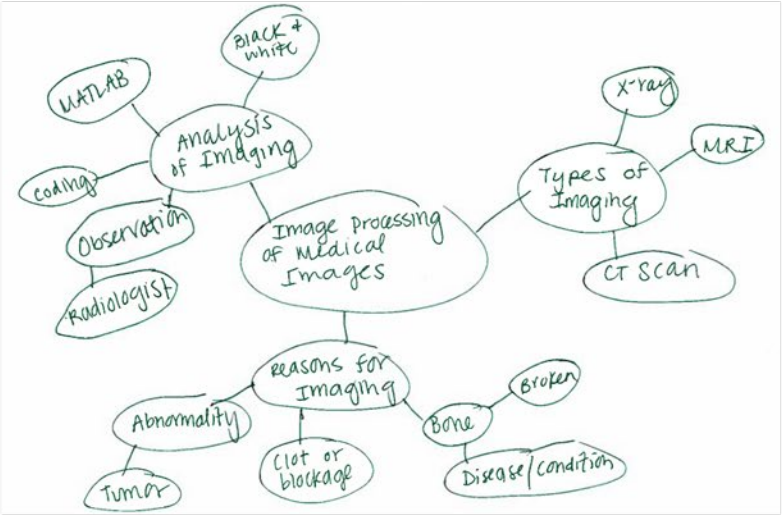
Alicia's more extensive coding background was evident in her pre-module concept map. She incorporated multiple foundational coding topics that were covered throughout the module and necessary for a high score in the comprehensiveness section of the rubric. In addition to foundational coding knowledge, Alicia included several concepts that related to the medical images used as inputs, another key idea covered in IntroCAD. Alicia's pre-module concept map did not include any concepts related to image processing methods or outputs, which was reasonable given that she had no prior experience with image processing.

In her mid-module map, Alicia demonstrated a large amount of growth in content knowledge related to image processing. Alicia's mid-module map included concepts related to image processing methods and properties of digital images, using field-specific language. This growth was similar to that of other students without Alicia's background in computer science, highlighting that a more extensive computer science background was not needed to grasp module concepts. Despite gains in comprehensiveness, Alicia had minimal gains in correctness because many of the connections contained within the map were naïve. For example, she connected "MATLAB," "matrices," "segmentation," and "manually coding" to the central bubble of "image processing," even though all these concepts were specific examples rather than general higher order concepts.

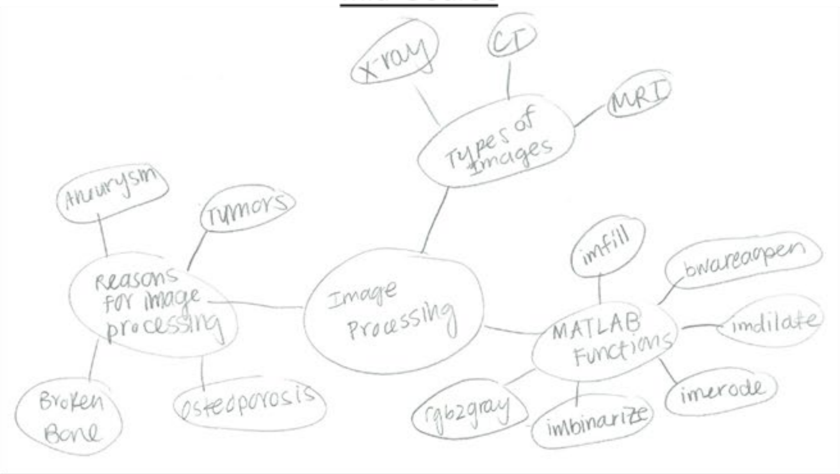
In her post-module map, Alicia demonstrated more extensive coverage of content and had more nuanced connections between concepts. Whereas her mid-module concept map demonstrated a naïve understanding of image processing by connecting specific examples to the central "image processing" bubble, her post-module concept map has some these specific examples branching off more general concepts (e.g., "Segmenting" is now a sub-concept of "Methods"). It is interesting to note that the organization of Alicia's concept map remained roughly constant throughout the module, increasing slightly in complexity mid-module the inclusion of a cross-link (though that cross-link is arguably invalid), and that the organization was that of a novice. This constant organization could suggest that Alicia's knowledge structure is subject to rapid change as she learns more about image processing and works toward a stable, highly networked knowledge structure.

Student 2: Tara. Tara demonstrated minimal growth in concept map holistic score from pre- to post-module (Figure 7-8). Tara was a third-year woman in the BME department. She had taken the introductory engineering programming course but had no other coding experience. Tara's primary motivation for taking IntroCAD was to increase her proficiency with MATLAB and was not related to the content material. In multiple short-response and Likert-type questions in the module pre-survey, Tara emphasized the importance of coding skills to her future career prospects in the BME industry.

Student 183 Pre-Course



Mid-Course



Post-Course

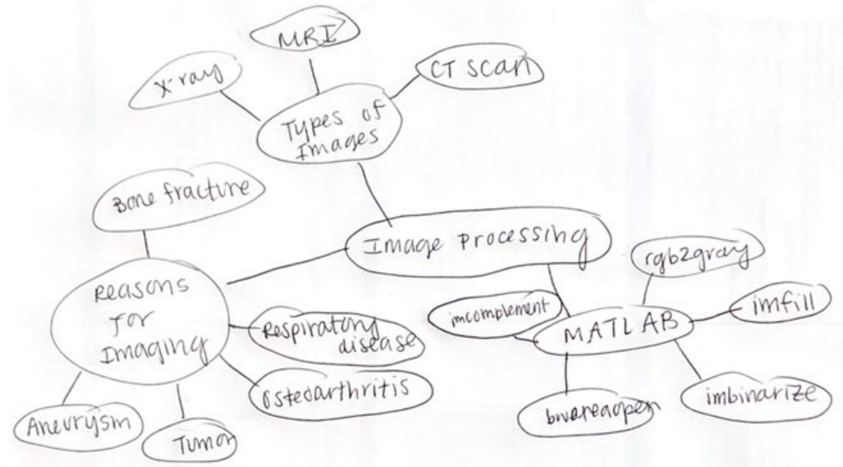


Figure 7-8. Concept maps from Tara, who demonstrated minimal growth in concept map holistic score pre- to post-module.

Tara's pre-module concept map incorporated multiple concepts related to clinical uses of medical images, including concepts like clinicians, imaging modalities, and disease states that would require imaging. The inclusion of these concepts could have been enabled by Tara's more advanced position in the degree program; many BME undergraduates have finished general requirements and start taking more discipline-specific coursework in their third year. Tara's pre-module concept map did not include any concepts related to image processing methods, properties of digital images, or analysis of digital images. Beyond the inclusion of several MATLAB-specific image processing functions, Tara's concept map did not develop much past her pre-module map. This lack of development in conceptual knowledge could have been due to a greater focus on coding skills acquisition rather than increased conceptual understanding of image processing during the module.

7.6 Discussion and Lessons Learned

In this chapter, the action research cycle was applied to improve the teaching of computer programming to BME students. We designed and implemented a four-week, one-credit module that combined traditional lectures, scaffolding, and PBL. All stages of the action research cycle, including research of previous implementations, design, implementation, and assessment are included in this chapter. Reflection and recommendations for future iterations can be found in Chapter 9. The structure of this module included two weeks of traditional lectures and labs with scaffolding and active learning exercises and two weeks of final project work time. Critical components of both scaffolding and PBL were incorporated into the module, the implementation of which can be seen in Table 7-8 (for PBL) and Table 7-9 (for scaffolding). Overall, students positively engaged with the material, educational strategies, labs, activities, and PBL.

Table 7-8 Implementation of PBL components into the final design project.

Critical Component of PBL	**Implementation**
Outcomes are well-defined	Project outcomes were defined based on rubric with clear specifications
Task is ill-defined to promote self-directed learning	Project focused on a body system and often required skills beyond what was covered in lecture and labs
Students work in cooperative groups to complete the task	Students worked in groups of 2-3
Instructors act as facilitators	Instructors asked questions and guided students toward resources rather than provide explicit instruction
Projects have real-life applications	Students used open-source radiology images from medical applications
Students engage in self-reflection	Students submitted a final report which critically evaluated their script's strengths and limitations

Table 7-9. Implementation of scaffolding into IntroCAD.

Critical Component of Scaffolding	**Implementation**
Contingency	Lectures, beginner- and advanced- level questions in lab, and discussions were tailored using survey responses and muddiest points; questions answered in class were based on responses to questions probing student background knowledge.
Fading	Instructors moved from providing explicit instruction to facilitating discussions; labs included progressively less detailed instructions ending with an open-ended final project.
Transfer of Responsibility	Students took more ownership of both discussions and project work as they transitioned from lectures and guided labs to discussions and an open-ended final project.

To apply the action research framework to this course, we assessed the implementation of our intervention with surveys and concept maps to demonstrate student gains in conceptual knowledge, perceived gains in skills-based knowledge, perceived applicability of skills, and high perceptions of instructors. Students indicated in the post-module survey that the module increased their knowledge in both image processing and computer programming (above 4.5 on a 5-point

Likert-type scale). Significant increases were found from pre- to post-module in confidence with image processing and use of computer programming to solve BME problems Students' attitudes toward instructor support and PBL had average scores of above 4 on a 5-point Likert-type scale, indicating that students had high perception of instructors and instruction techniques. The significant increase in concept map holistic scores from pre- to post-module time points shows that students gained conceptual knowledge from the module. Students in this module demonstrated clear gains in skills acquisition, self-efficacy, and beliefs in the applicability of knowledge, which suggests that our design, which had mini-lectures and labs preceding the final project, met our design goals and positively impacted students.

Chapter 8 Climate in STEM for LGBTQIA+ Individuals: Development of a Visibility Campaign.

This chapter has been adapted from a peer reviewed version of this work:

SE Hopper, C Tossas-Betancourt, P Walczyk, L Hirshfield. (2022, August) "The implementation and assessment of a social media initiative to increase visibility of LGBTQIA+ individuals in STEM (Research)," Paper presented at 2022 ASEE Annual Conference & Exposition, Minneapolis, MN. https://peer.asee.org/41056

8.1 Introduction

People who do not identify as heterosexual or cisgender, i.e., members of the LGTBQIA+ community or queer individuals[1], face numerous challenges when studying in science, technology, engineering, and mathematics (STEM) fields, which leads to a decreased retention of these students [228], [229], higher rates of closeting [230], and increased rates of mental health issues [231], [232]. These challenges were largely unexplored in the past, but there has been a recent increase in the number of studies focusing on LGBTQIA+ students in engineering and STEM [191]. These studies have found increased discrimination, harassment, and bullying towards

---

[1] Lesbian, gay, transgender, bisexual, queer, intersex, asexual and other sexual/gender minorities (LGBTQIA+) is an inclusive term we have decided to use to describe this community. Similarly, we will be using the term queer to describe any individual who identifies as something other than heterosexual or cisgender.

LGBTQIA+ students compared to non-LGBTQIA+ students, and a chilly climate in STEM, described as implicit or explicit disapproval towards queer identities [191], [232]–[235].

Queer theory, an application of critical theory described in Chapter 6, can be used to analyze the discourses STEM culture that enforce hetero- and cis-normativity, which is the legitimization of heterosexual and cisgender identities over queer identities, and exert power over queer individuals [236]. Using a queer theory lens, critical action research can be used to improve the climate in STEM for LGBTQIA+ individuals by dismantling these discourses. In this work, we used action research to develop a visibility campaign for those working and learning STEM at the University of Michigan. As opposed to the previous chapters of this thesis, we completed more than one action research cycle for this work, which can be seen in Figure 8-1. Specifically, we developed and implemented an Instagram-based visibility initiative that highlights the achievements of queer people learning and working in STEM at the university.

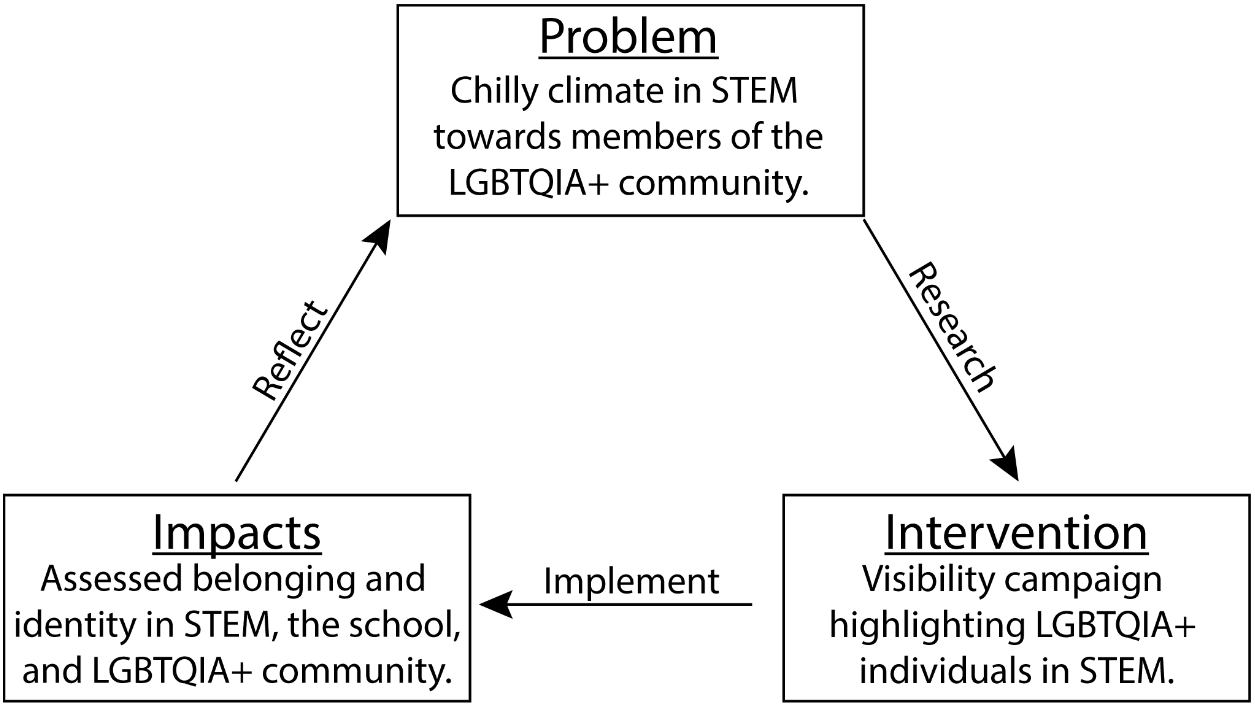


Figure 8-1. Action research cycle for improving the climate in STEM for members of the queer community.

The first action research cycle that was completed to improve the climate in STEM for queer individuals at the University of Michigan occurred in the Fall 2021 and Winter 2022 semesters, while the second action research cycle started at the end of action research cycle 1 and continued until Winter 2023 (Figure 8-2). Section 8.2 Action Research Cycle 1 includes all components of the first action research cycle, including a review of background literature pertaining to the climate for queer individuals in STEM, initial implementation of the visibility initiative, assessment, and reflection. In section 8.3 Action Research Cycle 2, you will find a discussion of the next action research cycle, starting from implementation of changes from the first action research cycle, assessment, and discussion of results. The reflection and suggestions for future iterations from the second action research cycle can be found in Chapter 9 – Discussion. In addition to assessing the impact of the visibility initiative, data was collected to characterize the climate in STEM for queer individuals and identify needs that can be address through the initiative. Data collection for the assessments occurred at three time points: at the launch of the STEM Pride at UM account (Fall 2021), at the beginning of the next academic semester (Winter 2022), and one year later (Winter 2023). A survey was distributed at all three timepoints and focus groups were performed at time point 3. The timeline of action cycle 1 and 2, along with the number of followers of the initiative on Instagram, participants in assessments, and numbers of assessment participants who were engaged in the initiative can be found in Figure 8-2.

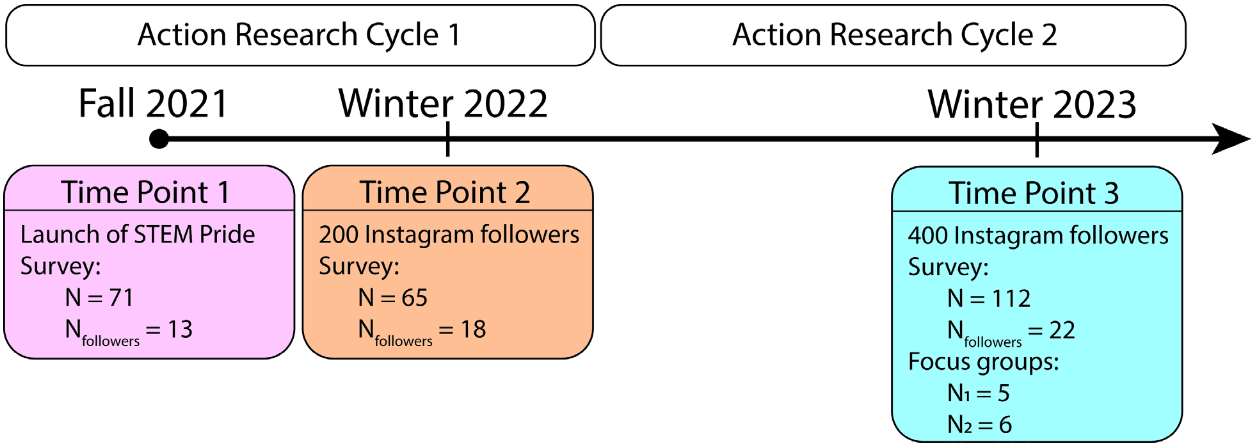


Figure 8-2. Data collection and action research timeline. Data collection occurred via surveys at three time points and via two focus groups at the last time point. Number of participants for each time point is included: N is the number of survey participants, $N_{followers}$ is the number of survey participants who actively engaged with the STEM pride initiative, N_1 is the number of participants at the first focus group, and N_2 is the number of participants at the second focus group.

8.2 Action Research Cycle 1

8.2.1 Previous Literature

Multiple discourses have been identified in STEM to enforce hetero- and cis-normativity including technical/social dualism [237], apoliticism [238], [239], hegemonic masculinity [233], [240], and bioessentialism [233]. Technical social dualism is the prioritization of topics in the technical domain over topics in the social domain, including communication, management, and conversations of diversity, equity, and inclusion (DEI) [241]. In this context, conversations concerning LGBTQIA+ identities and queer experiences are seen as inappropriate in STEM. Similarly, apoliticism is the belief that STEM disciplines are not influenced by politics to remain objective. Because of the politicalization of queer identities, apoliticism directly enforces hetero- and cis-normativity [235]. Hegemonic masculinity is the idolization of traditional masculine characteristics that devalues femininity [240]. While the implications of hegemonic masculinity for heterosexual women are clear, the implications for heterosexism are more complicated.

Hegemonic masculinity leads to a stigmatization of queer men, who are not seen as ‘real men,’ and legitimization of masculine-presenting queer and gender nonconforming (GNC) women, who may present with valued traits [233], [240], [241]. Bioessentialism is the belief in two genders based on sex-based characteristics [233]. This equates gender with biological sex and enforces the idea that transgender, non-binary, genderqueer, and other gender (trans+[2]) identities are illegitimate [233]. As mentioned in Chapter 6, it is important to acknowledge intersectionality and the cumulative disadvantage experienced by individuals who hold multiple marginalized identities. Few studies have focused on intersectionality with queer identities in STEM, including the intersection with disabled identities [185] and racial identities [242].

Previous studies have investigated how queer individuals in STEM navigate the chilly climate. Students often resort to compartmentalizing their queer identities to pass as heterosexual or cisgender, which causes feelings of isolation in their personal and academic lives and leads to lower retention [239]–[241]. Furthermore, this population has higher incidence of mental health issues, including anxiety and depression [232]. Few studies have characterized the effects of the climate in STEM on the social-psychological indicators discussed in Chapter 6, although a lower sense of belonging and difficulty developing STEM identity may be a cause of the observed negative outcomes. Stout and Wright (2016) showed that LGBTQIA+ students in computing had a lower sense of belonging and were more likely to consider leaving the field. Gay men have been shown to have a low sense of belonging in engineering [240]. Similarly in Haverkamp’s (2019) autoethnographic study, transgender and queer women discussed the culture of engineering decreasing their belonging in their field [230]. Many studies have proposed models for queer identity development, but few discuss the development of STEM identity of queer individuals

---

[2] In this study, we use trans+ to refer to anyone who identifies differently than their assigned gender at birth, including binary transgender individuals, non-binary, gender fluid, gender queer, etc.

[191]. Mattheis *et al.* (2020) developed a model of queer STEM identity based on STEM professionals, which can be seen in Figure 8-3 and includes the defining of queer identities, development of STEM identities, and navigating queer identities in STEM [243].

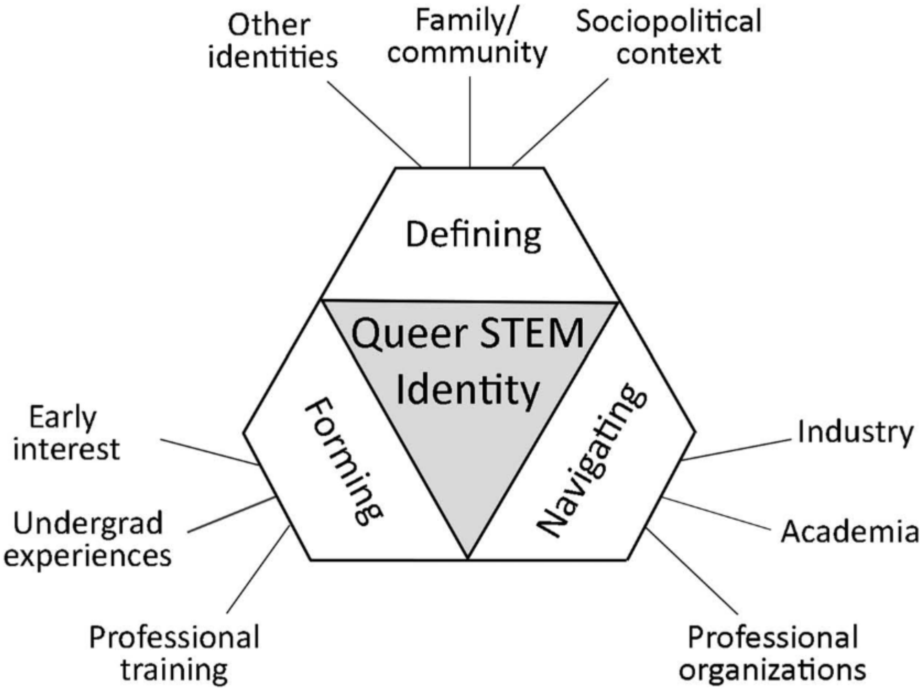


Figure 8-3. Queer STEM identity model from [243].

Another key aspect of queer theory is the focus on dismantling discourses in society that enforce hetero- and cis-normativity as opposed to queer individuals assimilating into society [236]. In this vein, numerous institutional changes should be pursued to positively impact the climate for LGBTQIA+ individuals in STEM, including expanding diversity-promoting and anti-discrimination policies to include queer identities, increasing campus resources for queer students, and Safe Zone or Ally training for faculty, staff, and students [191]. Visibility of queer individuals in STEM may promote a cultural shift in STEM towards being more accepting queer identities, in addition to providing positive role models, increasing peer support networks, decreasing feelings of isolation, and integrating STEM and queer identities, which may increase sense of belonging and support STEM identity development [185], [191], [234], [240], [243].

8.2.2 Implementation

To help increase visibility and create an online community for LGBTQIA+ individuals in STEM at the University of Michigan (UM), we created the ongoing STEM Pride at UM initiative, which features self-identifying LGBTQIA+ individuals in STEM on a public Instagram page (https://www.instagram.com/stemprideatum/). This initiative was largely inspired by 500 Queer Scientists, an online international campaign for increasing visibility of LGBTQIA+ individuals in STEM (https://500queerscientists.com/) [244]. Individuals highlighted on the page self-nominated through an interest survey that was emailed to various STEM and LGBTQIA+ organizations at UM. If students met criteria, i.e., being members of the LGBTQIA+ community and associated with STEM at UM, they were sent a form to input information for their posts. The posts focused on three aspects of individuals: (1) basics, including identities, pronouns, and hobbies; (2) academic interests, including field of study, future goals, and greatest achievements; and (3) experiences being LGBTQIA+ in STEM, including a notable event relating to their identity, advice for younger people interested in the field, and how they participate in mentorship. Two posts per individual were created from this information, which were sent to the individuals for approval and to request changes. An example post can be seen in Figure 8-4.

Theo Hopper

Trans-masc and Queer PhD Student in Biomedical Engineering

Pronouns: They/Them

My hobbies include baking, improv comedy, and reading.

I use computational modeling to study how diseases cause structural and functional changes to the cardiovascular system.

Biggest Accomplishment

While not a singular event, I think every step that gets me closer to my goals (going to graduate school, publishing papers, passing my qualify exam) is an enormous accomplishment, showing dedication, focus, and perseverance.

Future Goals

I would like to use my PhD to be a teacher, either at a smaller college or in a teaching-only position at a larger school.

Theo Hopper

Trans-masc and Queer PhD Student in Biomedical Engineering

Pronouns: They/Them

To younger LGBTQIA+ individuals interested in STEM...

I would tell them to follow their dreams, but make sure to build the queer support needed outside of classes and work. It is necessary (and fun) to have queer friends who understand you. I am very grateful to my queer support :)

How can you promote LGBTQIA+ visibility in STEM?

I help run this Instagram page :) I also am "unapologetically queer" in every space I go to, which includes being visible and talking openly about queer issues.

I participate in mentorship by...

I was a queer mentor for one semester through the spectrum center and interact with undergraduate students through teaching and research.

A notable experience in STEM related to being LGBTQIA+ is...

I haven't had any experiences with anyone being homophobic or transphobic in STEM. However, in many of the spaces I inhibit, I am the only trans/GNC person, which gets lonely and exhausting.

Figure 8-4. Example post from the STEM Pride Instagram. Post one (TOP) focuses on the individual's interests, goals, and accomplishments. Post two (BOTTOM) focuses on the individual's relationship with the LGBTQIA+ community.

Posts were designed to incorporate multiple aspects of the individuals featured, not only focusing on their work in STEM. Individuals are allowed to describe their identities any way they see fit and are also encouraged to include any other identities that are important for them. This is aimed to allow for flexibility in identity descriptions and promote an intersectional view. Furthermore, we do not ask individuals to discuss how they have struggled because of their queer identities, rather we employ a strengths-based approach by focusing on accomplishments and service. To not ignore the difficulty of being queer in STEM and decrease feelings of isolation of followers who are struggling in STEM because of their identities, we ask individuals to describe a notable experience in STEM related to their queer identities, which individuals can interpret as a positive or negative experience. In addition to featuring individuals from UM, STEM Pride at UM also aimed to increase broader LGBTQIA+ awareness. For example, weekly posts were made about notable and historical LGBTQIA+ individuals in STEM for LGBTQIA+ history month.

Finally, STEM Pride at UM hosted various events for community building or education. Community building activities attempted to connect individuals at the intersection of the queer community, STEM, and UM and included a coming out day celebration and support group surrounding anti-LGBTQIA+ legislature. These events were advertised on the STEM Pride Instagram account and to queer and STEM groups on campus. Education-based events attempted to bring awareness of general queer issues to allies, and included allyship training, which was an overview of LGBTQIA+ identities and proper use of pronouns presented by the campus LGBTQIA+ center, and open office hours for non-community members to ask questions about fostering a safe environment.

8.2.3 Assessment

Sense of belonging and identity are key social-psychological factors for success and well-being in an academic environment, which can be used as indicators for assessing the impact of DEI initiatives and the integration of individuals into social settings [145]. In this work, we use sense of belonging and identity to investigate individuals' navigation in their STEM field, queer community, and university. An individuals' sense of belonging and identity are impacted by the climate and values of these communities, their intersections, and the global societal context, which can be visualized in Figure 8-5. These constructs are also not stagnant and change with time dependent on individual and societal factors.

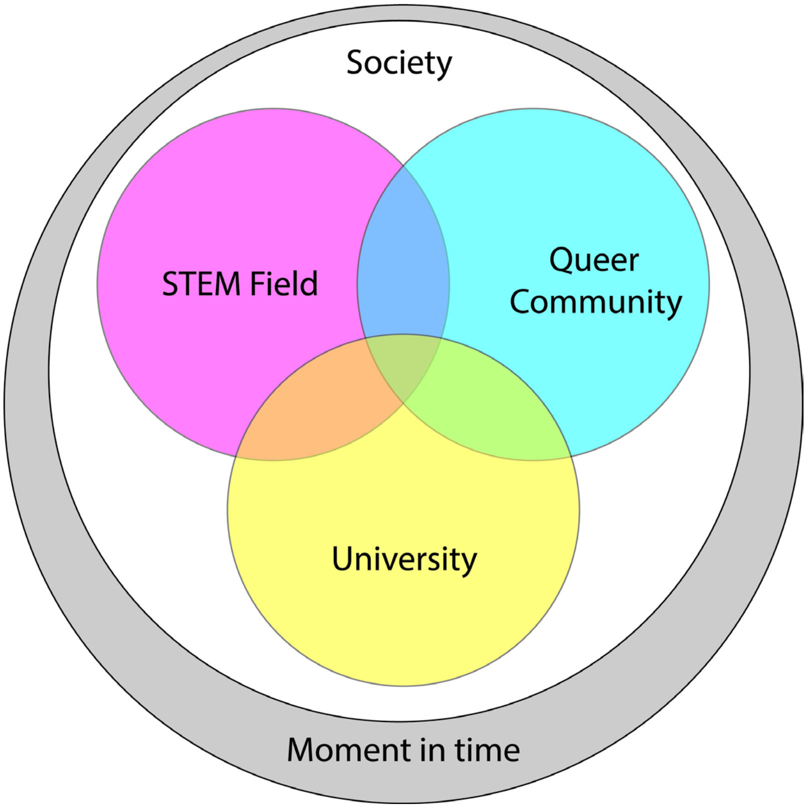


Figure 8-5. Communities that affect sense of belonging and identity for queer people in STEM at a university. In this study, we assessed belonging and identity to the STEM field, queer community, and the university.

Surveys were used to characterize the climate in STEM for, identify needs of, and assess the impacts of the STEM pride initiative for LGBTQIA+ individuals at UM. To characterize the climate in STEM and identify needs, a survey was developed to assess sense of belonging and identity to STEM field, queer community, and the university. To assess the impacts of STEM pride, survey participants who engaged with the initiative were asked 5-point Likert-type scale questions about the perceived impact of STEM pride on belonging and identity and open response questions about the impact and considerations for being featured. Focus groups also contained a discussion about the initiative and its impact on individuals. Data collection was approved by the University of Michigan's Institutional Review Board (HUM#00206614).

Questions included in the survey to assess sense of belonging and identity were adapted from previous work [148], [245]–[247]. The survey used 5-point Likert-type questions, ranging from *strongly disagree (1)* to *strongly agree (5)*. All Likert-type questions distributed in the survey are included in Appendix B. Participants were also asked demographic information, including their race/ethnicity, sexual orientation, gender identity, college of study, and position within the university. For each of the demographic questions, participants were allowed to select more than one response or opt to not answer. The survey was distributed on the STEM Pride at UM Instagram page and various LGBTQIA+, STEM, and diversity-focused organizations at UM and was active for three-week periods.

8.2.4 Data Analysis

Survey participants were removed from analysis if they did not complete the Likert-type scale question related to belonging and identity or if they did were not members of the LGBTQIA+ community in STEM at UM. 24 questions were included in our survey, and these questions were categorized in 6 constructs: (A) belonging with the university, (B) belonging with STEM field, (C) belonging with the queer community, (D) identity with the university, (E) identity with STEM field, and (F) identity with the LGBTQIA+ community (Appendix B). Responses to Likert-type questions were averaged within each construct, which were used to perform statistical analyses. Statistical inference testing was performed with analysis of variance (ANOVA) testing between constructs of belonging and identity and time. All analyses were performed using the R statistical computing software.

Statistical clustering was used to investigate the impact of other identity-based factors on participants' sense of belonging and identity at each time point. Participants who did not complete the demographics portion of the survey were removed from clustering analysis. To ensure that the data was clusterable, the Hopkins statistic was estimating using the get_clust_tendency function from the R package factoextra [248]. Dissimilarity matrices were created for each time point using

Gower's distances from the daisy function in the R package cluster [249], [250]. Clusters were generated from the dissimilarity matrices using the partition around medoids (PAM) algorithm via the pam function from the cluster package [251], [252]. Optimal number of clusters was determined based off of the largest average silhouette width, a metric of the distance between an observation and the nearest cluster of which it does not belong, outputted from the pam function [252], [253]. Once appropriately clustered, chi-squared analysis was used to determine which demographic factors (race, sexual identity, gender identity, identification as transgender, academic school, and position at the university) and statistical inference testing, in the form of Student's t-test or ANOVA based on the number of clusters, was used to determine which values of belonging and identity were significant between the clusters.

8.2.5 Demographics of Participants

Demographics of survey participants for the first two timepoints are described in Table 8-1. Demographics of survey respondents were similar between the timepoints. At both timepoints, the majority of respondents were white; bisexual, pansexual, or queer; cisgender; women; in engineering; and students with approximately equal number of undergraduates and graduates. Due to errors in the wording of the question to assess gender identity, the number of self-identifying transgender individuals is not available for the first and second timepoint. For the sake of appropriately representing participants' gender identities, participants who identified as transgender men and women are included as their gender identity and as binary transgender in Table 8-1. The demographics of survey participants from timepoints 1 and 2 who actively engaged with the STEM pride initiative can also be seen in Table 8-1. Most survey participants did not engage with our initiative (unaware of it: 46 and 39; aware of it but do not follow: 12 and 7 at timepoints 1 and 2 respectively).

Table 8-1. Demographics of survey respondents at timepoint 1 (pink) and timepoint 2 (orange) and survey respondents who actively engaged with the STEM Pride initiative (right).

		Respondents		Respondents- followers	
		Time 1	Time 2	Time 1	Time 2
Race/Ethnicity	White/Caucasian	55	45	11	15
	Eastern Asian	8	15	2	2
	Indian	3	0	0	0
	Hispanic/Latinx	4	8	0	1
	Black	2	3	0	0
	Native American	2	1	0	0
	Middle Eastern and North African	1	2	0	1
	Mixed Race	2	8	0	1
Sexual/Romantic Identities	Gay	18	19	3	4
	Lesbian	13	16	5	7
	Bisexual	26	20	5	8
	Pansexual	6	9	2	2
	Asexual	12	6	1	2
	Aromantic	6	1	1	0
	Queer	23	11	5	5
	Straight	0	1	0	0
	Androsexual	0	1	0	0
	Polyamorous	0	1	0	0
	Confused	1	0	0	0
Gender Identity	Man	21	18	2	3
	Woman	40	35	12	14
	Binary transgender (man or woman)	5	3	1	0
	Non-binary/third gender	14	8	1	1
	Prefer not to specify	3	3	0	0
	Genderqueer	1	1	0	0
	Agender	1	1	0	0
	Gender-expansive	0	1	0	0
School	Art and Design	1	1	0	0
	Engineering	47	47	7	15
	Environment and Sustainability	2	1	1	0
	Kinesiology	0	1	0	0
	Literature, Science, and the Arts	11	9	1	9
	Medicine	6	3	3	2
	Nursing	2	0	1	0
	Pharmacy	1	2	0	0
	Public health	1	0	0	0
Position	Undergraduate Student	35	31	3	6
	Graduate Student	31	29	10	9
	Post-Doc	1	1	0	0
	Faculty	2	3	0	3
	Alumni	1	0	0	0

8.2.6 Results – Climate in STEM

No significant changes in belonging and identity with the STEM field, queer community, and university were found between the first and second time points (Figure 8-6); however, statistically relevant differences were observed between constructs of belonging and identity at each time point. At both timepoints 1 and 2, sense of belonging with the queer community was higher than either the STEM field or the university. No significant differences in identity constructs were observed at timepoint 1; however, at timepoint 2, sense of identity with the queer community was higher than with the STEM field.

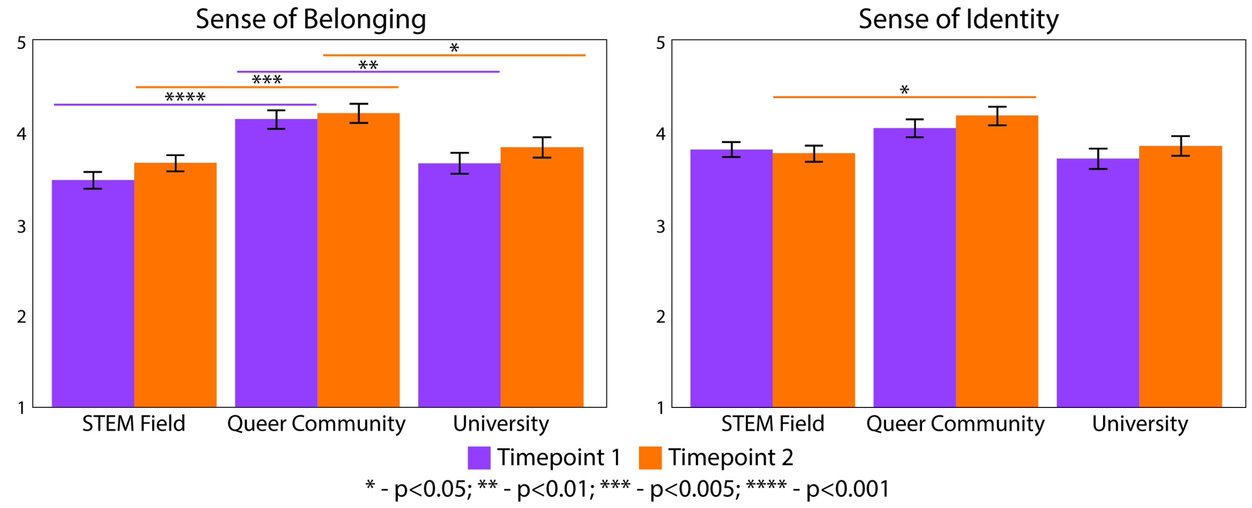


Figure 8-6. Mean responses to Likert-type scale questions about sense of belonging (LEFT) and identity (RIGHT) with STEM field, queer community, and university for the first (purple) and second (orange) timepoints. P-values from inference testing are indicated with stars.

Data from both timepoints were determined to be clusterable based on a Hopkins Statistic greater than 0.5 [254]. Two clusters were identified for both timepoints 1 and 2. Differences in sense of belonging and identity between the clusters for both timepoints can be seen in Figure 8-7. At time point 1, the only significant factor between clusters was position at the university. Cluster 1 (N=37) being composed of mostly undergraduate students (34) with 2 graduate students and 1 postdoc, and cluster 2 (N=31) being composed of mostly graduate students (29) with 2 faculty members. Cluster 2 (graduate students) exhibited a significantly lower sense of belonging with STEM field and University and a significantly lower sense of identity with the university than cluster 1 (undergraduate students). No differences were observed in terms of sense of belonging or identity with the queer community. At timepoint 2, significant factors between clusters included sexual identity, gender identity, and position at the university. Cluster 1 (N=28) was composed of mostly gay (18) male (16) graduate students (20), while cluster 2 (N=36) was composed of mostly bisexual (14) or lesbian (12) female (27) undergraduate students (26). Cluster 1 (gay male graduate students) exhibited a lower sense of belonging and identity with the university than cluster 2 (bisexual or lesbian female undergraduate students).

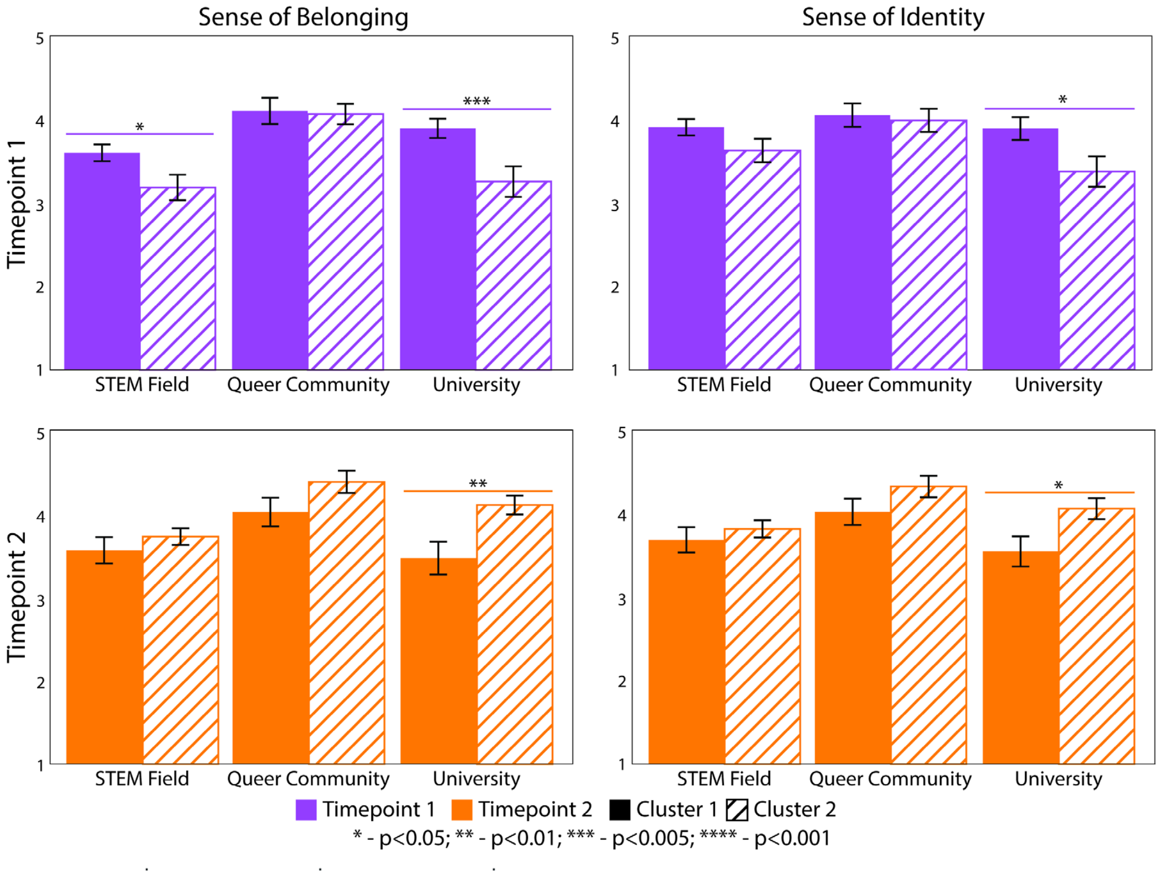


Figure 8-7. Comparison of sense of belonging and identity between identified clusters for timepoint 1 (top; purple) and timepoint 2 (bottom; orange). Timepoint 1 was separated into two clusters with undergraduate students (cluster 1 – solid bar) and graduate students (cluster 2 – striped bar). Timepoint 2 was separated into two clusters with gay male graduate students (cluster 1 – solid bar) and bisexual or lesbian female undergraduate students (cluster 2 – striped bar).

8.2.7 Results – Impacts of STEM Pride

Survey participants who were engaged in the STEM Pride initiative, i.e., who indicated they were followers of or wanted to be featured on the STEM Pride at UM Instagram page, had a relatively positive (mean >3.5) perception of the initiative's impact on their sense of belonging with the STEM field, queer community at the university, and university and sense of identity in the STEM field, queer community at the university, and queer people in their field Figure 8-8. There were no significant differences in the perceived effect of STEM Pride at UM between the timepoints.

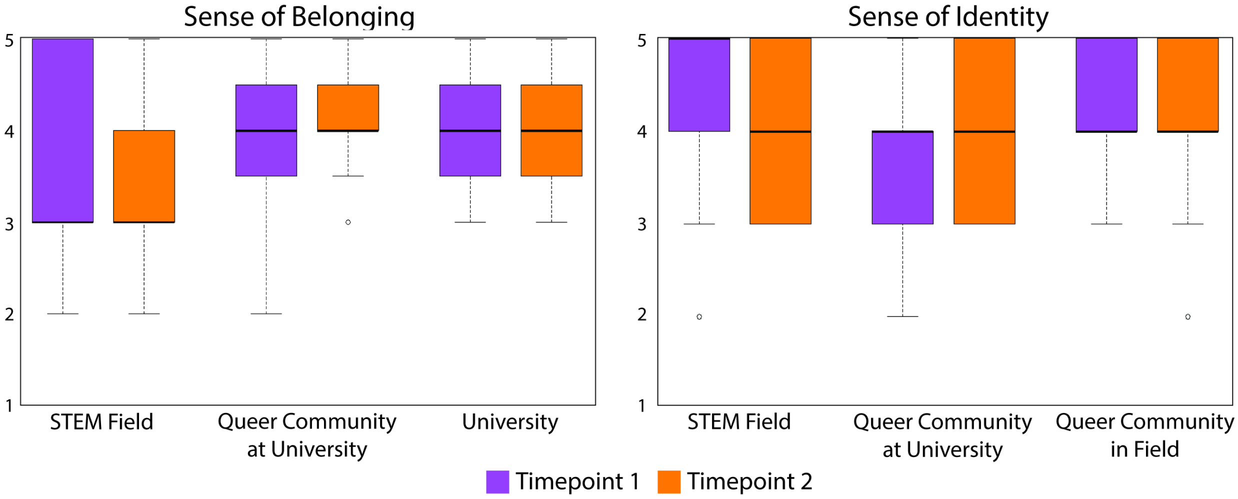


Figure 8-8. Perceived impact of STEM pride on sense of belonging and identity from survey participants who actively engaged with the initiative ($N_{followers}$ = 13 and 18 for timepoints 1 and 2 respectively) at timepoint 1 (purple) and 2 (orange).

When describing the impact of STEM Pride at UM, participants at both timepoints mentioned the importance of visibility and overall positive perception of the initiative. For example, a participant mentioned at the first timepoint: "It is nice to see people in the LGBT[QIA+] community be vocal about who they are. I struggle with feeling like I will [not] be accepted and have a tendency to try to pass as straight as the default in unfamiliar situations." A participant in the second timepoint said: "It feels great to see other members of the LGBTQIA+ community in

STEM… That visibility is very important to me and has made me feel less isolated." While another participant at the second timepoint said: "I have periodic reminders that I'm not alone and that's very important." In addition to showing the general positive perceptions of participants, these quotes highlight that STEM Pride decreased participants' sense of isolation and feelings of needing to cover or pass in STEM.

Participants did not want to be featured on the Instagram page due to a variety of reasons, including privacy concerns and not being out to everyone in their lives, which confirmed the pressure to compartmentalize LGBTQIA+ identities within STEM. Multiple participants expressed disinterest in being featured due to not feeling "queer enough" since they were in opposite sex relationships or identified as queer, bisexual, or pansexual. On the other hand, participants who expressed interest in being featured highlighted that they wanted to be visible for others in the community, make connections with others, and show that STEM and LGBTQIA+ identities "are intertwined, not just separate".

8.2.8 Reflection

The survey data from timepoints 1 and 2 highlights the importance of initiatives to increase sense of belonging and identity in STEM and at universities. Because the scope of this study is to use critical action research to identify needs of and improve the climate for queer individuals in STEM, we did not collect data for people who did not self-identify as members of the queer community and cannot make claims concerning differences between members of the LGBTQIA+ community in STEM and their peers. However, the significantly higher sense of belonging towards the queer community compared to their STEM fields and university exhibited by survey participants suggests that the climate in these realms could be improved to benefit these individuals. Furthermore, results of clustering analysis identify subpopulations in STEM that can especially benefit from initiatives to increase sense of belonging and identity, including graduate students and gay men.

Despite no global effect in sense of belonging or identity, individuals who actively engage with the STEM Pride initiative have positive perceptions of it. In addition to relatively high answers to Likert-type scale questions concerning the impact of the initiation on belonging and identity, participants described the initiative reducing their feelings of isolation and pressures to pass as straight or compartmentalize their identities in STEM. Feeling isolated in STEM has been previously correlated to decreased sense of belonging in STEM and decreased emotional well-being [186]. As mentioned previously, the pressure to compartmentalize or hide their queer identities has multiple negative effects on queer students, including reducing sense of belonging and being correlated to not persisting in the field [241].

Because the majority of survey respondents being unaware of the initiative, increasing the number of queer people in STEM at the university who actively engaged with the STEM Pride initiative was identified as the largest potential improvement at the end of the first action research cycle. Additionally, survey respondents who engaged with the initiative were majority white, cisgender, queer women, so we also aimed to increase efforts to promote intersectionality and broaden the diversity of followers and people who were featured. We also wanted to improve survey distribution, because the majority of survey participants were also white, cisgender, queer women. Anecdotally, the most successful method for advertising the initiative and survey was through student organizations for queer individuals in STEM, which likely skewed the survey and initiative participants towards those with an active queer community. Additionally, we were not satisfied with the amount and type of information collected to characterize the climate in STEM and identify needs of the queer community. Through quantitative assessment of sense of belonging and identity, we were not able to identify components of STEM culture that impact queer individuals and can be improved through the STEM Pride initiative. Finally, participants identified issues with the survey question to assess gender identity, which erroneously made a distinction between binary transgender and cisgender individuals.

8.3 Action Research Cycle 2

8.3.1 Implementation

Based on the areas of improvement identified through the first action research cycle, we implemented changes to the STEM Pride at UM initiative and data collection methods to better characterize the climate in STEM. To improve general awareness of the initiative, we increased advertising efforts and specifically promoted to general STEM and university-based groups. We also hosted more events and collaborated with other queer in STEM organizations. Additional community building events included trivia with the university out in STEM (oSTEM) chapter and attending a community-based pride celebration, which connected queer individuals in STEM at the university but also supported and connected to the local queer community. To connect to the global queer community, we fundraised for Transanta, an organization that provides gifts to transgender youth without support or financial stability (https://www.transanta.com/). In addition to educational events for allies about issue concerning the general queer community, we hosted events for members of the queer community to learn about experiences of those with intersectional identities in the queer community. For example, we celebrated Asian American and Pacific Islander (AAPI) heritage month with a journal club discussion about the issues facing queer AAPI individuals in K-12 STEM education (ref. [255]).

8.3.2 Assessment

To improve the ability of survey participants to describe their gender identity, an additional question was included for individuals to indicate if they identified as transgender. The categories included in the gender identity question were also expanded. The survey distribution methods were all improve by sending to specific STEM departments, academic advisors, and more organizations not specifically associated with queer identities. In addition to the survey to assess sense of belonging and identity of queer individuals in STEM at the university, focus groups were performed to gain a deeper knowledge of an individual's perception of the climate and techniques they use to navigate their identities. Focus groups were advertised on the STEM Pride Instagram page and to LGBTQIA+, STEM, and diversity-focused organizations at UM. Two virtual focus groups were scheduled based on researcher availability at different times and days of the week to maximize participant availability. Focus groups were held virtually and were semi-structured; topics included perceptions and experiences related to queer identities in their department, field, and university; methods to build queer community; advice they would give to queer individuals considering joining their department, field, and university; impacts and suggested improvements to STEM Pride; and what their considerations were for being featured. With participants' consent, transcripts were generated during the focus group using the Zoom software and were immediately deidentified by removing participants names and any other personal information. The researcher who led the focus group also read through the transcript immediately to correct any obvious mistakes made by the caption generating software.

8.3.3 Focus Group Analysis

After each focus group, transcripts were analyzed using an inductive thematic approach and queer theory as a lens, specifically analyzing the ways participants describe, interpret, and explain the marginalization of queer identities in STEM. Queer STEM identity theory was also used as a framework to examine how participants navigate their queer identities in STEM. Using the online software Taguette, themes were identified from focus group transcripts related to STEM climate, effects of individuals, and identity navigation techniques. Main themes that emerged in the focus groups included *climate*, *outness*, *visibility*, *emotion*, *community*, *research*, *validation*, *STEM Pride*, *trans+ issues*, *DEI efforts*, *intersectionality*, and *education*. In this study, we define queer visibility as interpersonal expressions and observations of queerness, while outness is the intrapersonal decisions to display or discuss queerness with others. Next, subcodes were identified from portions of the transcripts associated with each theme. For example, subthemes associated with *community* were *strategies to build*, *university organizations*, and *effects on well-being*. Connections between themes and subthemes were identified.

8.3.4 Researcher Positionality

It is important to acknowledge the identities of researchers and how they may influence the research process. In this study, I, Theo Hopper, held focus groups and analyzed qualitative data. I am a trans-masculine, queer, white, neurodiverse, abled-bodied, graduate student in biomedical engineering. I knew many of the focus group participants personally, which may have influenced the responses that participants felt comfortable sharing.

8.3.5 Participant Demographics

Demographics of survey participants for timepoint 3 can be seen in Table 8-2. The amount of survey respondents almost doubled from timepoints 1 and 2 (N = 71, 65, and 112 at timepoints 1, 2, and 3, respectively). However, the demographics are similar to timepoints 1 and 2 with the majority of participants being white; bisexual, pansexual, or queer; cisgender; women; in engineering; and students with approximately equal number of undergraduates and graduates. The majority of participants were unaware of the initiative (70) or were aware but did not follow (20). The demographics of survey participants who followed or wanted to be featured can also be seen in Table 8-2 (right). The demographics of survey participants who engaged with the initiative were also similar to timepoint 1 and 2; however, the number of undergraduates increased (3, 6, to 11 at timepoints 1, 2, and 3 respectively) and the number of transgender and non-binary individuals increased (1, 1, to 12 at timepoints 1, 2, and 3 respectively).

Table 8-2. Demographics of survey participants and participants who engaged with the initiative at timepoint 3.

		Respondents	Respondents - Followers
Race/Ethnicity	White/Caucasian	81	18
	Eastern Asian	14	1
	Indian	2	0
	Hispanic/Latinx	9	2
	Black	5	0
	Native American	1	0
	Middle Eastern and North African	2	2
	Mixed Race	6	0
	Prefer not to Specify	4	1
Sexual/Romantic Identities	Gay	21	3
	Lesbian	10	3
	Bisexual	40	8
	Pansexual	12	1
	Asexual	19	6
	Aromantic	9	4
	Queer	33	10
	Biromantic	1	0
	Arospec	1	0
	Confused	1	0
Trans	Yes	17	3
	No	89	14
	Prefer not to specify	5	2
Gender Identity	Man	36	3
	Woman	49	14
	Non-binary/third gender	21	9
	Prefer not to specify	1	0
	Agender	7	2
	Gender Fluid	5	0
	Questioning or unsure	12	4
School	Engineering	79	12
	Kinesiology	1	1
	Literature, Science, and the Arts	9	2
	Medicine	18	5
	Nursing	2	1
Position	Undergraduate Student	58	11
	Graduate Student	47	10
	Faculty	3	0
	Staff	1	1

The assigned alias, school, position at UM, identities, and pronouns of focus group participants can be seen in Table 8-3. To protect their anonymity, participants were assigned aliases, and specific departments within schools are not included. Queer identities are included in Table 8-3 as well as any other identities that participants discussed during the focus groups. All but one of the focus group participants were in engineering (10/11) and most were graduate students (8/11). Only 2 of the participants were men, whereas 4 were women, and 5 were non-binary or in the process of questioning their gender.

Table 8-3. Demographics of focus group participants. Participants were assigned aliases to protect their identities.

Assigned Alias	Pronouns	Identities	School	Position
Alex	She/her	Bisexual; person of color	Engineering	Graduate student
Ben	He/him	Gay	Life sciences	Staff
Casey	She/they	Asexual, non-binary	Engineering	Graduate student
Daniella	She/her	Queer	Engineering	Graduate student
Erin	She/they	Asexual, non-binary	Engineering	Graduate student
Faith	She/her	Queer	Engineering	Graduate student
Gene	He/they	Queer, non-binary/questioning gender	Engineering	Graduate student
Harry	He/him	Queer	Engineering	Undergraduate student
Isa	She/they	Queer, non-binary/questioning gender	Engineering	Graduate student
Jaime	She/her	Queer	Engineering	Graduate Student
Kai	They/them	Queer, non-binary, intersex	Engineering	Post-doc

8.3.6 Survey Results – Climate in STEM

No significant changes in sense of belonging and identity with the STEM field, queer community, and university were found with time; however, the trend of all constructs was to decrease from timepoint 2 to timepoint 3. Figure 8-9 shows all constructs of belonging and identity at all three time points. Similar to timepoints 1 and 2, sense of belonging with the queer community is significantly higher than the STEM field or university. Sense of belonging with the STEM field is additionally significantly lower than the university at timepoint 3. Similar to sense of belonging, sense of identity with the queer community was significantly higher than either STEM field or university.

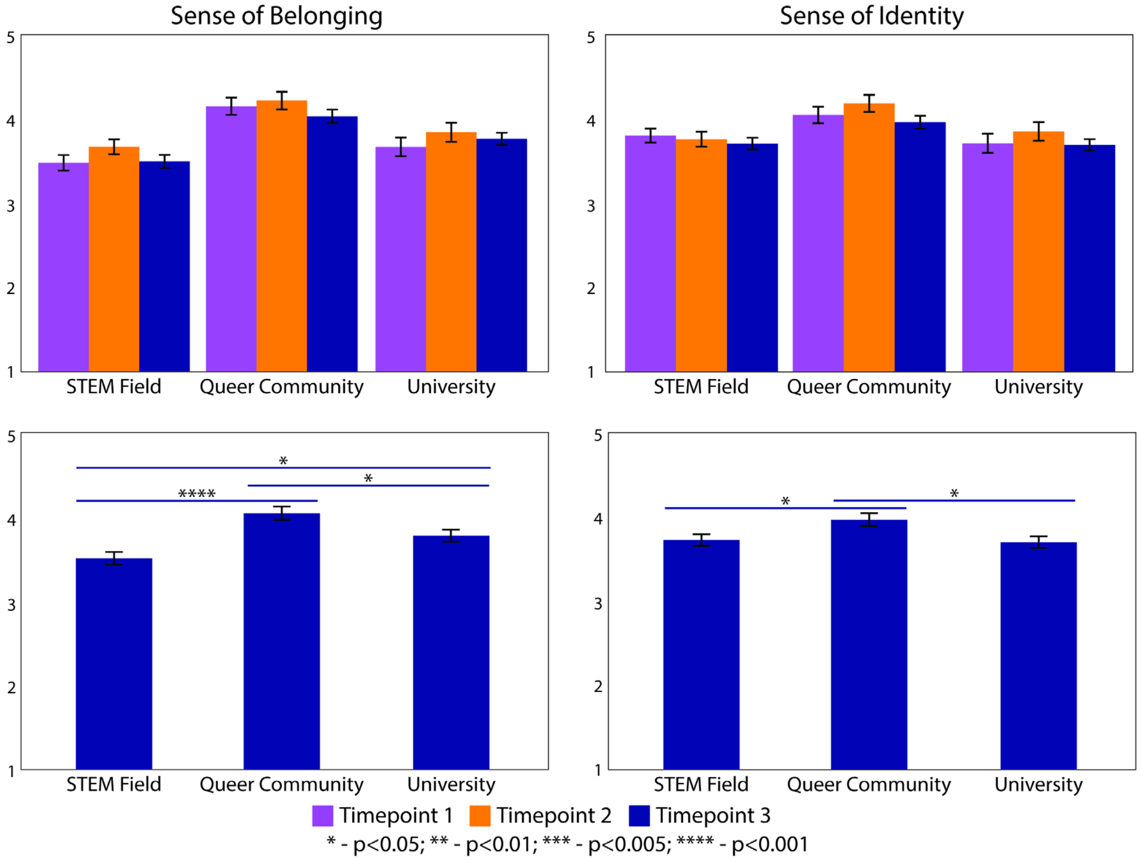


Figure 8-9. Sense of belonging and identity with STEM field, queer community, and university for all three timepoints (TOP) and for timepoint 3 with significant differences between constructs indicated (BOTTOM).

Data from timepoint 3 was determined to be clusterable based on a Hopkins Statistic greater than 0.5 [254]. Three clusters were identified based on silhouette width. Significant factors between the clusters include race, gender identity, sexual identity, position at the university, and school. The demographics of each cluster can be seen in Table 8-4. For brevity, clusters will be discussed in terms of the demographic factors with the largest differences between groups; cluster 1 was identified as female graduate students in medicine, cluster 2 was identified as engineering undergraduate students, and cluster 3 was identified as gay male undergraduate students in engineering. Differences in sense of belonging and identity between the clusters can be seen in Figure 8-10. Cluster 2 (engineering undergraduate students) had significantly higher sense of belonging and identity with the university compared to cluster 1 (female graduate students in engineering) and significant higher sense of belonging and identity with the queer community and sense of identity with the university than cluster 3 (gay male undergraduate students in engineering). Cluster 1 (female graduate students in engineering) had significantly higher identity with the queer community than cluster 3 (gay male undergraduate students in engineering).

Table 8-4. Significant demographics of the three clusters identified for timepoint 3.

		Cluster 1 (N=23)	Cluster 2 (N=56)	Cluster 3 (N=30)
Race/Ethnicity	White/Caucasian	16	44	17
	Eastern Asian	1	6	7
	Indian	0	1	1
	Hispanic/Latinx	0	3	5
	Black	3	0	2
	Native American	0	1	0
	Middle Eastern and North African	0	2	0
	Mixed Race	0	1	5
	Prefer not to Specify	3	1	0
Sexual/Romantic Identities	Gay	0	0	18
	Lesbian	3	6	0
	Bisexual	10	22	6
	Pansexual	2	8	3
	Asexual	2	5	4
	Aromantic	2	3	1
	Queer	7	19	3
	Biromantic	0	0	1
	Arospec	0	1	0
	Confused	0	0	1
Gender Identity	Man	0	9	27
	Woman	15	30	1
	Non-binary	3	11	2
	Prefer not to specify	1	0	0
	Agender	2	4	0
	Gender Fluid	1	4	0
	Questioning or unsure	2	6	2
School	Engineering	5	47	22
	Kinesiology	1	1	0
	Literature, Science, and the Arts	0	5	4
	Medicine	14	0	4
	Nursing	0	2	0
Position	Undergraduate Student	1	49	8
	Graduate Student	22	5	20
	Faculty	0	2	1
	Staff	0	0	1

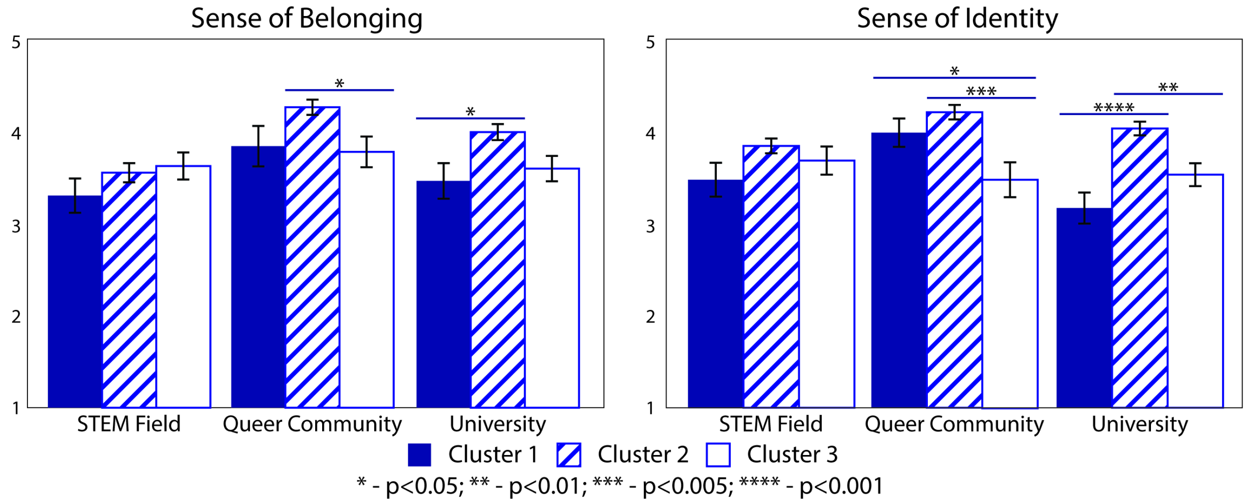


Figure 8-10. Sense of belonging and identity with STEM field, queer community, and university for each of the three clusters identified for time point 3.

8.3.7 Focus Group Results – Climate in STEM

From analysis of the focus groups using a queer theory lens, we gained an understanding of how aspects of STEM culture enforce cis- and hetero-normativity and marginalize queer individuals. Participants in the focus groups described their experiences in STEM similarly to previous studies that report a 'chilly' climate for queer individuals. Furthermore, conversations led to insights about what social structures in STEM culture contribute to this climate, and participants alluded to many discourses that have been identified previously from literature to negatively affect queer individuals, including apoliticism [238], [239], technical/social dualism [237], and hegemonic masculinity [233], [240], as well as an overemphasis on objectivity and work-devotion. Participants described experiences of enforcement of cis- and hetero-normativity and marginalization of queer identities that result from these discourses. Finally, we were able to gain information related to the effects of this climate on individual well-being and strategies used to navigate queer identities in STEM. An overview of the insights gained from focus group analysis can be seen in Figure 8-11. Below you will find the general impressions of STEM climate for queer and trans+ individuals (climate in STEM), perceived discourses from STEM culture (identified discourses), evidence for the resulting marginalization of queer individuals (experienced marginalization), effects on individual (effects on individuals), and strategies used to navigate queer identities (identity navigation).

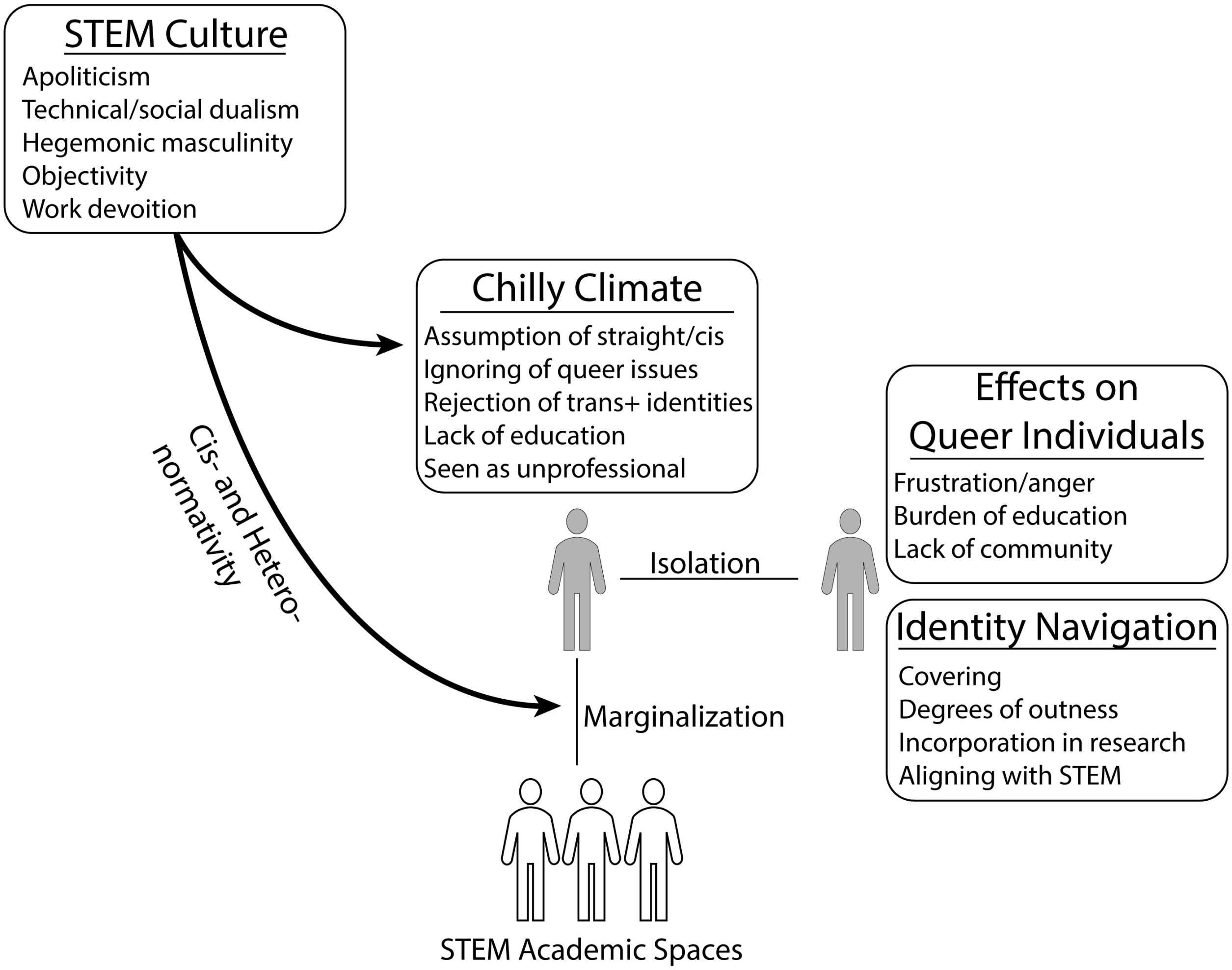


Figure 8-11. Visual representation of focus group analysis with queer theory and queer in STEM identity framework. Aspects of STEM culture enforce cis- and hetero-normativity, causing a chilly climate in STEM for and marginalization of queer individuals (gray), resulting in isolation, negative personal effects, and the need to navigate competing identities.

Climate in STEM. Focus group participants described the climate in STEM for queer individuals similarly to previous studies [191], [238], [241], [256]. Specifically, participants described aspects of a ‘chilly’ climate, which was not explicitly homophobic but was also not explicitly friendly towards queer identities. Isa (she/they) described her perception of the climate in STEM as:

> *"I've never felt like I was like ever unsafe, or like wasn't supported necessarily as a queer person. But just like things here and there that … feels a little off…"*

Isa discussed feeling not completely comfortable in STEM surrounding her queer identities despite not being able to identify specific incidences of harassment or discrimination. Participants mentioned transgender and non-binary identities being explicitly rejected in STEM through incidences of microaggressions and harassment. Casey (she/they) described these experiences in STEM surrounding her trans+ identities as:

> *"What I run into is more people seeing my pronouns, [which] gets them confused… the issues I run into aren't so much with the LGB, it's more so with the T."*

Casey mentions a general sense of confusion surrounding the use of their pronouns and also described a specific incident of someone interrogating them about their trans+ identities. Isa (she/they) described experiencing microaggressions by often being assumed to be a woman, especially in engineering spaces consisting of mostly women, which negated their non-binary identities. Kai (they/them) described experiencing transphobic harassment surrounding their trans-focused research and an incident of being physically unsafe on a university bus. Furthermore, participants discussed issues related to trans+ identities being excluded from conversations about increasing the gender diversity in STEM. Faith (she/her) described the only instances that she experiences resistances to queer inclusion while doing DEI work being related to incorporating trans+ issues in these conversations:

> *"I have felt friction in like with queer identity with students… once we start to talk about like spaces for women also including trans and nonbinary people."*

In addition to DEI-based work, participants mentioned the exclusion of trans+ individuals in demographic portions of papers and surveys.

Identified Discourses. Participants described their queer identities as being relegated by others in their departments as not important for or *"not an issue"* in STEM, relating to apoliticism and the technical/social dualism. Regarding technical/social dualism, fields in STEM that are less quantitative were described as being more friendly towards queer identities. Ben (he/him), who works in an interdisciplinary STEM field and has experiences with various departments, describes this explicitly when discussing STEM departments that are more queer-friendly:

> *"My sense is that when something becomes more 'quantified,' there tends to be an academic culture that is less emotional supportive."*

In this quote, Ben connected friendliness towards queer identities to being emotionally supportive, a social skill, which he viewed as at odds with heavy quantitative work. While not explicitly mentioning quantitative work, other participants connected acceptance of queer identities with "non-traditional" engineering spaces, which included spaces for engineering education research and excluded defense- or industry-focused research.

In addition to the root causes of apoliticism and technical/social dualism, participants related the overemphasis on objectivity and work-devotion as discourses that discourage discussions of queer identities in engineering fields specifically. Isa (she/they) mentioned objectivity in engineering culture by saying:

> *"There's a very specific engineering like way of doing things, you know, we're doing engineering work. It's objective. There's nothing really outside of that."*

Harry (he/him), the only undergraduate who participated in our focus groups, attributed the workload of his engineering program to the decentralization of queer identities:

> *"We're all so busy that I think that whole work taking precedent over the rest of your life is kind of, I guess, kept everything quiet that isn't related to engineering work."*

Through the overemphasis of objectivity and prioritization of engineering work over identity, participants perceived that anything not connected to engineering, including queer identities, were not welcome in their work or learning spaces.

Hegemonic masculinity in STEM culture was commonly alluded to in focus groups. Participants discussed feeling more comfortable in spaces with better gender parity, which was influenced by queer and gender identities. Multiple participants described specific STEM spaces, including the 'traditional' engineering research areas discussed above and field sciences, that they experienced or observed incidences of misogyny, which was used as a signal for that space also not being open to queer identities. Gene (he/they) described this succinctly, noting that *"sexism is the yellow flag before homophobia."* Gene also described a lack of representation in their department with other masculine-presenting queer people:

> *"There are a lot of queer people that are engaged with as women in my department, but not a lot of like queer men… as someone who presents as a guy and like some of my struggles with masculinity in the queer community like, there's not a lot of solidarity."*

Previous studies have investigated the impacts of hegemonic masculinity for queer men, who may experience an especially hostile environment in STEM if they are feminine-presenting [240], [256], which may contribute to the lack of other out queer men in Gene's department.

Experiences of marginalization. Participants discussed experiences in STEM that exhibit the enforcement of cis- and hetero-normativity and marginalization of queer identities. Multiple participants discussed being assumed to be straight and cisgender in STEM spaces and connected these to not displaying typical hallmarks of queer culture in their appearance, not being in a same-sex relationship, and not holding leadership positions in queer focused organizations. Some participants discussed being dissuaded from incorporating traditionally queer aspects into their appearance as it was viewed as unprofessional in their fields, which is evidence for the marginalization of queer identities from STEM professions. Casey (she/they) discussed this by saying:

> *"…like dying your hair, cutting it off, being – sort of – visibly more queer… is not considered professional. So when I go to conferences, I think I am sort of subconsciously trying to tone it down…"*

Alex (she/her) also perceived the reverse effect by the imposed definition of professionalism for women being contrary to how she experiences femininity:

> *"I guess I don't know where the external pressure is coming from… but like as a woman who needs to go to conferences and dress professionally, there certain expectations of what I'm supposed to wear."*

Later in the conversation, Alex included wearing makeup and having long hair as some expectations for professional dress. Participants also mentioned that these definitions of professionalism are shifting to be less exclusive of typical expressions of queerness. Alex mentioned that although she felt as if these expectations are still present in her field, they are being enforced less stringently and less explicitly. Faith (she/her) mentioned not facing backlash in her department for a more queer-aligned haircut (an undercut), which she might have expected in the not-so-distant past. As mentioned previously, spaces in STEM that are viewed as especially queer friendly are also viewed as 'non-traditional,' which is further evidence of marginalization of queer identities.

Effects on individuals. The chilly climate for and marginalization of queer individuals in STEM had negative effects on focus group participants. Many participants described being frustrated or angry at the state of STEM for queer issues. Faith (she/her) described being frustrated in her field in general, based on the lack of representation and inequity:

> *"I think some technical fields like are very, you know, white, straight cis-passing or cis-presenting men's field, who are predominantly from wealthy backgrounds and have access to technology from their youth. It's pretty frustrating in [my engineering field] for me."*

Participants also mentioned being angry because of a lack of inclusivity efforts surrounding queer issues, experiences of transphobia or harassment, and double standards based on gender. Kai (they/them) described instances of transphobia and harassment at conferences, which lead to them not attending conferences in the future, missing potential networking and career advancement opportunities. Specific instances of marginalization lead to participants feeling *othered* or *tokenized*. For example, Alex (she/her) described efforts for queer inclusivity in her workspace making her feel othered as the only out queer person in the space. Similarly, Casey (she/they) felt tokenized because of having to repeatedly discuss an issue regarding the queer community with people in her field, because they were the only openly queer person people knew in their field.

These instances of being othered or tokenized relate to feelings of isolation described by participants. Many participants mentioned feeling as though they are the only queer person or one of few queer people in their departments, which connected to a difficulty building queer community. Isa (she/they) discussed needing to invest emotional energy in order to build queer community as a graduate student in STEM:

> *"It's like on us. The onus is on us to like find our community and navigate through it."*

Harry (he/him) also mentioned needing to devote energy to find queer community. He attributed joining university organizations to decreasing his feelings of isolation:

> *"Even if it feels like you're completely alone, you'll find someone that can help you out. You just… might just have to reach out a little though. It's like I thought I was like one of like 5 queer people department. And then, like I joined so many student orgs I found like, oh, these like 20 people I did not know were queer. in fact, are. All I had to do was say something."*

However, the use of university resources for building queer community was less successful for graduate student and post-doc participants, many of whom described feeling out of out of place at queer events that involved many undergraduates, being unaware of the resources available to them, or attending events that did not have sufficient turnout for them to feel comfortable mingling with others. Some of these participants used resources not associated with the university to build queer community, including online spaces and community organizations. Other participants highlighted the importance of visibility for building queer community through creating connections based on communication with one open queer individual in their field. Participants described the benefits of building queer community in STEM for reducing feelings of isolation and increasing support systems.

The isolation and marginalization of queer identities in STEM also caused individuals to experience a burden of education and performing DEI work regarding queer issues. Faith (she/her), who was active in DEI for her department, described this burden:

> *"Anything that I want anyone to be educated about is something that I have to educate them on. Regardless of whether it's something within my experience, or just like, you know, something that's generally in the queer community that I would like them to respect. I have to be the educator, because most people most like faculty and staff in my department do not know unless I tell them."*

In addition to highlighting the responsibility assumed by Faith because of isolation and a lack of knowledge surrounding queer issues, this quote demonstrates that idea that people not in the LGBTQIA+ community require education before providing respect to LGBTQIA+ issues. In addition to within their departments, other participants discussed needing to educate others on DEI committees about queer issues, which suggests a lack of focus on queer identities and educations within DEI spaces. This idea was echoed by other participants describing DEI efforts by the university as performative and uninformed. This burden of education and performing DEI work for queer issues is particularly harmful for the participants who are students, who do not have much power in their departments to elicit change and are reliant on those with power in their departments for professional and academic development. Participants discussed how the DEI work they are engaged with leads to them being viewed as *"troublemakers,"* a negative perception that could harm their future prospects in their departments.

Identity Navigation. Participants described strategies they used to navigate their queer identities in STEM. As they were often assumed to be cisgender and heterosexual, some individuals described tactfully disproving this assumption based on their comfort in a space. Jaime (she/her) describes this navigation strategy and the effects it has on her:

> *"I feel like I almost drop back into playing straight at times just to fit in and not make anyone else feel awkward… and I almost have to make the decision of like am I gonna be me or am I just gonna be half of me today?"*

In this quote, Jaime mentioned that she uses the comfort of others as a reason covers her queer identity, but she also mentioned her own comfort and avoiding emotionally unsafe situations at another point in the focus group. The negative impacts of covering can be seen through Jaime's description, as she felt as though she was unable to share her full self with others. The burden of education was also a factor that influenced the decision to cover their identities, especially for trans+ participants. Erin (she/they) discussed incorporating the need to educate others about their pronoun use in their decision to be open with others:

> *"It's whatever you're willing to like, educate for, like a lot of people try to be non-offensive, but they're not going to put in… work."*

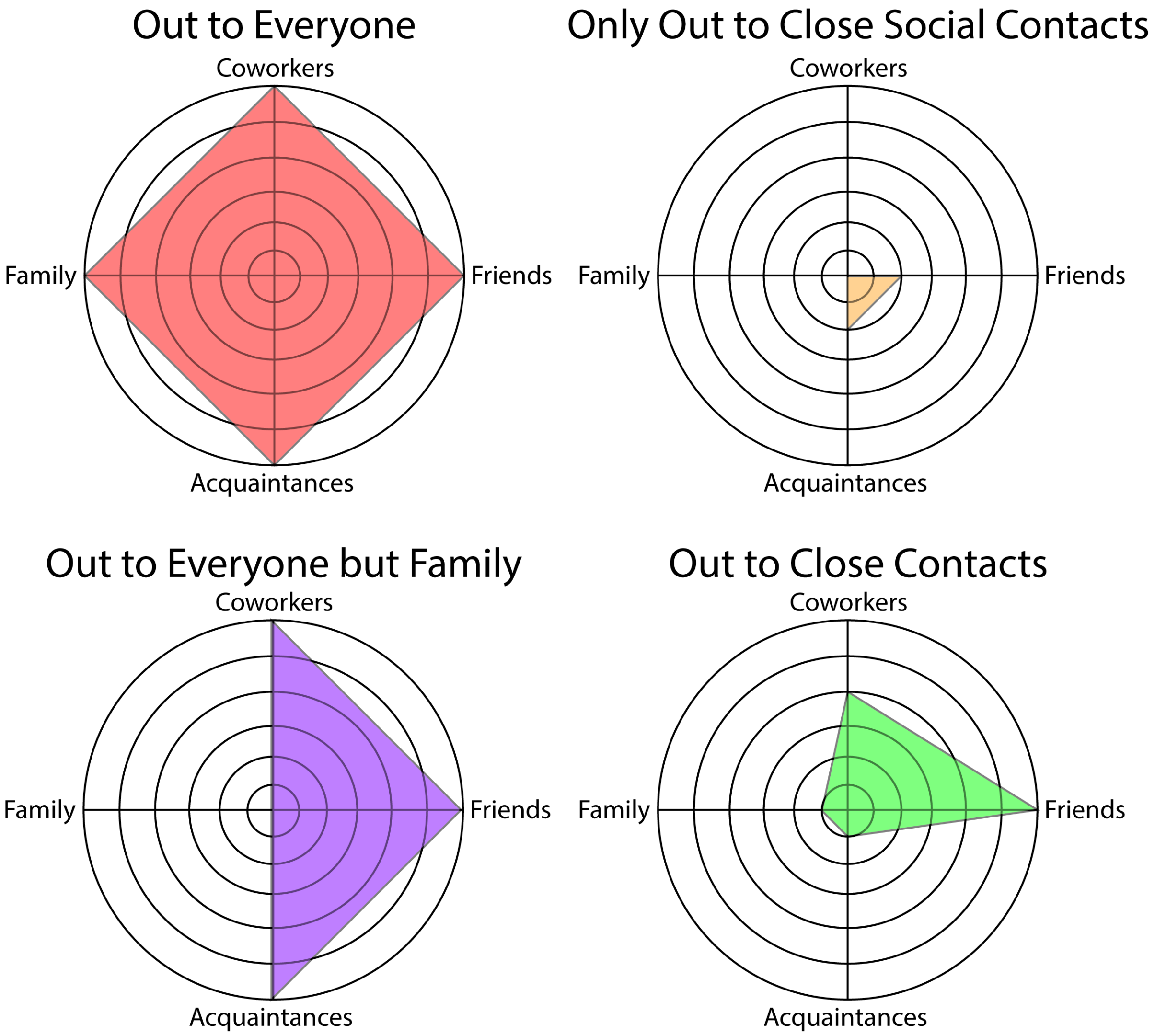
Erin mentioned the emotional work associated with educating others affecting their decision to conceal their identities. She described this work being transferred to queer individuals when it is not accepted by those unaware of how to properly use her pronouns.

Participants described their covering tactics using words such as *"negotiation," "optimization,"* and *"strategic,"* which implies a process of taking in information about their environment and analyzing potential outcomes associated with sharing their identities. In focus group conversations, specific signs were mentioned that signaled safety to participants, including explicit displays of acceptance of the queer community, e.g., rainbow stickers, pride flags, and displaying pronouns. Visibility was a big safety signal for participants as well, who described gaining insight about the acceptance of queer identities based on the experiences of others. Isa (she/they) alluded to effects of seeing other queer people being open in a space and explicit displays of acceptance while describing the positive impacts of a space they and an open coworker created by being open about their identities:

> *"We want[ed] to put an inclusive pride flag in our space, just to be there, so students know they can like hopefully feel comfortable and be themselves here fully… They come into our space and are like 'no, I feel really comfortable…' because, like I don't know, we provided that space for them just by being ourselves."*

Through being visible about queer identities, Isa was able to safety signal to others leading to increased comfort and feelings of not needing to cover identities. Participants also mentioned ways in which they started conversations surrounding their queer identities. In the absence of explicit signs of acceptance of the queer community, multiple participants suggested subtly incorporating queer issues in conversations with coworkers and people in power to gather additional information about views towards the queer community before disclosing their identities. This further identifies strategic and deliberate methods used by the queer individuals in STEM to ensure emotional and physical safety.

In addition to displaying covering behaviors, i.e. concealing of queer identities in spaces, our participants displayed varying degrees of outness based on social realm, i.e., the people in their lives who they felt comfortable disclosing and not concealing their identities [257]. The different groups of people that participants considered included in discussions of their outness were friends, acquaintances, coworkers/academics, and family. All of the participants were completely out to close friends, but some participants discussed not sharing their identities with groups of friends depending on social context or closeness. We consider acquaintances to be people who participants interact with infrequently in the workspace or social spheres. For participants, being out to acquaintances again depended on social context and also a comfort discussing their queerness openly in spaces with people they do not know everyone or degree of visibility of queerness to others. Coworkers or academics includes anyone participants work or learn closely with, including peers, professors, administrators, or supervisors. Many of the participants in our focus groups were out to most of their coworkers, although Erin (she/they) was not out to anyone in their work sphere and was uncomfortable discussing their queer identities in the workspace, based on potential negative repercussions to their social or professional opportunities. They discussed coming out to those they know will not be offended or unaccepting in their lives. We include both close or immediate and extended in our discussions of family. Many participants did not discuss their outness with family during the focus groups, although Alex (she/her) discussed the impact of her families' Middle Eastern culture on her ability to share her queer identity with them. A visual representation of the realms that participants considered in outness conversations can be seen in Figure 8-12 including examples based on themes observed from conversations with participants.


Out to Everyone

Only Out to Close Social Contacts

Out to Everyone but Family

Out to Close Contacts

Figure 8-12. Visual representations of groups of people that participants discussed considering in terms of being out: coworkers, family, friends, and acquaintances with four examples based on participants: out to everyone, out to close social contacts (i.e., not to family or coworkers), out to everyone but family, and out to close contacts (i.e., out based on emotional closeness regardless of sphere).

Participants who discussed being completely out or open to others in their workplace described the benefits of being out. Ben (he/him) viewed outness as a necessity:

> *"Be who you are. There will be consequences to it that you cannot anticipate. But the consequences of not being who you are will be poor mental health and a crisis down the road. So choose what you want to prioritize, but the reality of it, you have to survive, which to me is being out."*

Ben acknowledged the potential negative effects of being out, but he viewed them as less significant than the negative effects of not being out – mental health crises. For Gene (he/they) being out is associated with comfort:

> *"I think for me like, because, like, I'm comfortable being, you know, loud. Amongst other things, it's like easy for me to just be kind of very loudly myself."*

Gene's default is to be vocal about their identities and covering would require additional energy. Participants also discussed altruistic reasons they wanted to be out, mainly for improving visibility for others in the queer community, especially if their identities were traditionally underrepresented in the LGBTQIA+ community. For example, Alex (she/her) discussed the importance for her to be out as a woman of color. Furthermore, outness was viewed as a form of resistance. Ben (he/him) discussed the benefits of making people uncomfortable through visible queerness, which he believed would lead to greater acceptance of queer individuals in the future.

In addition to strategically being out or covering their queer identities in STEM, some participants discussed trying to incorporate their queer and STEM identities. This was often done through connecting their STEM work to the queer community, for example by researching gender-affirming healthcare or the climate in climate for LGBTQIA+ individuals. Isa (she/they) described the impact of being able to incorporate their queer identities into their STEM work:

> *"I feel like it gives more room to bridge like 2 parts of yourself, to some at least, my experience, like 2 parts of yourself, that don't always necessarily, and so to have them align is like a very beautiful thing to me."*

Isa discussed feeling unable to bring her whole self to STEM work because of the climate towards her queer identities, similar to Jaime (she/her) above, which she mitigates through using her STEM research to help the queer community. Performing DEI work to help the queer community in STEM and academia was another way that participants combined their queer identities with their professional lives and build queer community. Most participants of the focus groups were active in DEI for their departments or the university.

The act of completely redefining their identities to better align with their STEM fields was not observed in most of our participants, although covering or not being out in any STEM space may suggest some evidence of this. However, Gene (he/they) described feeling a deeper connection to their STEM field than their queer identities:

> *"I identify more as an engineering than as queer."*

Although not a conscious navigation tactic, putting more weight in engineering identities may help Gene cope with misalignment of STEM and queer identities. Ben (he/him) also described secondhand experiences of others overcompensating in STEM because of their queer identities, leading to burn out.

8.3.8 Impacts of STEM Pride

Similar to time points 1 and 2, participants who actively engaged with the STEM had a relatively positive (mean >3.25) perception of the initiative's impact on their sense of belonging with the STEM field, queer community at the university, and university and sense of identity with the STEM field, queer community at the university, and queer community in their field

(Figure 8-13). No significant differences were identified between responses for timepoint 3 and the other two time points.

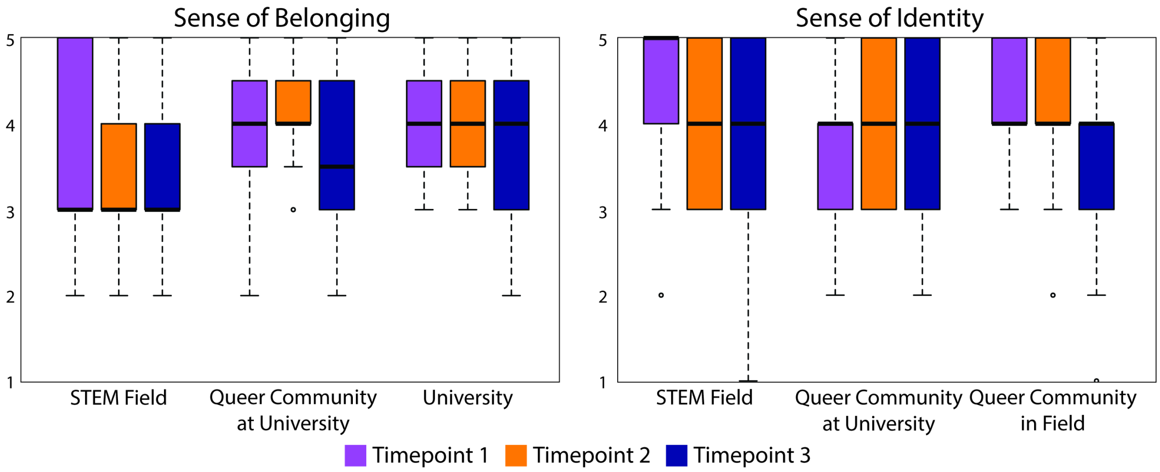


Figure 8-13. Perceived impact of the STEM Pride initiative on metrics of sense of belonging and identity at all three time points.

In focus groups and survey open responses, participants discussed the importance of the STEM pride initiative for decreasing feelings of isolation, building community, and increasing comfort expressing queer identities in STEM spaces. Daniella (she/her), a follower of the initiative, discussed the impact of the visibility of queer people via STEM pride on decreasing her feelings of isolation:

> *"I might not be meeting [the people featured] in person, but it is nice to see like these people exist, and we probably are like higher in numbers that I would think if I didn't follow this account."*

Isa (she/they), who frequently looks at the Instagram page to look at the features, discussed how seeing more queer people in STEM makes them feel like they have more people in their queer in STEM community, even if they do not interact with the people featured at all. Participants who were featured on the Instagram page also discussed the impact of the page for decreasing their feelings of isolation. In addition, Kai (they/them) discussed how their queer community in STEM increased as a result of their feature:

> *"I got supportive messages and felt more comfortable in my building."*

Finally, a follower from the survey discussed the importance of the account for them feeling comfortable exploring their gender in STEM:

> *"[STEM Pride Instagram account] inspired [me] to fully explore my gender; if the trans and non-binary people featured can be out in engineering, so can I."*

The reasons that participants included for wanting to be featured on the page were similar to timepoints 1 and 2, including increasing visibility of specifical identities and fields and decreasing the feelings of isolation of others. For reasons they did not want to be featured, participants discussed being afraid, not knowing what labels to apply to themselves, and not being out to everyone who might see it.

Focus group participants also gave suggestions for improving the STEM Pride initiative. Some participants mentioned that they are not frequently on Instagram, so they recommended expanding to other dissemination methods. Daniella (she/her) connected this to previous discussions of varying levels of outness as well:

> *"I feel like it's really challenging to just use one platform, because some people might be more comfortable being out in the workplace, but not around their like outside of workplace spheres. And then some people might be the opposite, like they're more comfortable being out to their friends and family outside of the workplace, but they don't want to be out to like that workplace."*

Expanding the initiative to be more than just on Instagram would allow more individuals to engage based on what social spheres they are out to. Gene (he/they) recommended that we connect to broader queer history and culture more on our page:

> *"Maybe trying to sort of provide some aspect of a cultural touchstone. In addition to like, you know, promoting queerness in like an individual way."*

Finally, Erin (she/they) suggested incorporating a way for individuals to reflect upon how their identities have changed over time in their featured. They recommended this so that followers could see that queer identities are *"fluid."*

8.4 Discussion

In this work, we described the implementation of the critical action research cycle to improve the climate in STEM for members of the LGBTQIA+ community. Based on previous studies, we developed a social media initiative that highlights the accomplishments in and connections with the queer and STEM communities for individuals working and learning STEM at the University of Michigan. In addition to featuring individuals, the initiative also celebrates queer history and hosts events for community building and education. We completed two action research cycles to develop and improve the initiative from Fall 2021 to Winter 2023. Assessment strategies were utilized to characterize the climate in STEM for and understand needs of the queer community at the university and to determine the impacts of the initiative. A survey was distributed at three time points (Fall 2021, Winter 2022, and Winter 2023) to quantify participants' sense of belonging and identity with their STEM Field, queer community, and university. Based on limitations in data collection identified during the first action research cycle, focus groups were included at the third timepoint to gain a deeper understanding of an individual's perception of the STEM climate, strategies for navigating their queer identities in STEM, and perceptions of the initiative. After the first action research cycle, we also made changes to the initiative, including attempting to increase awareness, engagement, connections with the general queer community, and awareness of intersectional issues. Reflection from the second action research cycle, including a discussion of the impacts of these improvements and suggested adjustments for future iterations can be seen in Chapter 9.

Through surveys and focus groups, we were able to gain an understanding of the climate for queer individuals in STEM at the University of Michigan. At all three survey collection timepoints, participants had a higher sense of belonging with the queer community than their STEM field or university. At timepoint 3, the sense of belonging with STEM field was also significantly lower than with the university. Similar trends were observed in respect to the sense of identity, which was higher than field identity at timepoints 2 and 3 and higher than university identity at timepoint 3. These results identify the need to improve STEM and university climate to be more welcoming to queer individuals. Clustering techniques were used to identify what demographic factors were important for participants' sense of belonging and identity. The important factors identified included position at the university, gender and sexual identity, school, and race. Specifically, we found that graduate students had significantly lower field and university belonging than undergraduate students. The results also indicate that gay men in STEM had lower sense of belonging and identity in the queer community than other identities, which may be a result of the demands of hegemonic masculinity in STEM fields and have been previously suggested from qualitative studies of this population [240], [256]. However, future studies should be performed with larger and more diverse sample sizes to investigate which of the identified factors are important for belonging and identity and which were determined to be statistically significant based on the demographic makeup of our population. For example, race was identified as a significant factor for timepoint 3 although the majority of participants in each cluster identified as white.

The descriptions of the STEM climate by focus group participants were similar to previous studies describing marginalization of queer individuals in STEM. Through thorough comparisons to previous studies, we can examine the ways in which STEM culture has remained stagnant or improved for queer individuals. In one of the earliest discussions of gay and lesbian students, Lopez and Chim (1993) describe the experiences of a gay man, Greg, who is pursuing an undergraduate degree in mechanical engineering [258]. Many aspects of the experiences Greg described 30 years ago was also observed in our participants today, including marginalization of queer issues because of social/technical dualism, overemphasis of objectivity, and instructors' lack of awareness [258]. However, many of the explicitly homophobic experiences described by Greg, including the use of slurs and association of queerness as 'deviance,' were not experienced by our participants. Furthermore, we identified ways in which the STEM climate has changed in the past 12 years by comparing our findings to Cech and Waidzunas (2011) [241]. One particular difference is that Cech and Waidzunas (2011) reported their participants often hearing phrases like 'that's so gay' used disparagingly in STEM spaces, which was not reported by our participants. Their participants who identified as bisexual, pansexual, or queer described STEM peers being confused by their sexual orientations, which was also not reported by our focus group participants, who mostly identified with these orientations [241]. Finally, the high number of trans+ participants in focus groups allows us to specifically examine the experiences of this population in STEM, which has rarely been studied previously [191]. The experiences of our trans+ participants were similar to the experiences described in Haverkamp *et al*. (2019), who also reported explicit rejection of their identities in engineering spaces.

By analyzing focus groups with the lens of queer theory, the aspects of STEM culture that exert power over queer individuals were identified, which cause the chilly climate for and marginalization of these individuals. Many of the discourses we identified were consistent with previous studies, including hegemonic masculinity, technical/social dualism, and apoliticism. Participants also discussed experiences related to the overemphasis on objectivity and work-devotion contributing to them not being able to fully express their queer identities in STEM spaces. Queer theory also emphasizes the need to dismantle the forces in culture to improve the environment for individuals. Our analysis is also important for the identification of potential areas of improvement that can be addressed with future DEI initiatives, discussed in Chapter 9.

Finally, focus group analysis identified the effects of the chilly climate on individuals and strategies they use to navigate their queer identities in STEM. Participants described multiple negative effects of the climate in STEM, including feelings of anger and frustration, isolation, difficulty building community, and being burdened with educating non-queer individuals. Common strategies for navigating identities included covering, selective outness, incorporation of queer issues in STEM research, and overemphasis on STEM identities. While examining the impact of these individual effects on well-being and academic achievement are outside of the scope of this project, previous studies have found that queer individuals have lower retention and higher rates of mental health issues in STEM [191], [239], [240], which may be because of the negative effects and negotiation techniques mentioned by participants.

8.4.1 Limitations

Because the sample size of the assessment methods was small and homogeneous, especially concerning race, ethnicity, and STEM field, the insights gained regarding the climate in STEM at the University of Michigan may not be representative of students who hold other marginalized identities. Additional focus groups should be organized to gather additional information regarding the experiences of more diverse participants. Specifically, most of our focus group participants were white graduate students in engineering. It is also important to consider that the goal of this project was to characterize and improve the climate in STEM at the University of Michigan, so the insights gathered may not be applicable to other institutions. This is especially true given the impact of societal factors outside of academia and STEM, including the legislative and cultural restrictions imposed on queer individuals that vary regionally.

Chapter 9 Reflection and Recommendation for Changes to STEM Education Practice

9.1 Discussion

. Research-based pedagogies, including active learning techniques, have been shown to improve student learning and self-efficacy [172]. Incorporating these strategies can be daunting, especially without prior experience. Practical action research can be used to optimize the implementation of research-based teaching strategies in the classroom through iterative improvement based on student outcomes. In Chapter 6, we used practical action research to improve educational strategies for teaching computer programming to biomedical engineering (BME) students. Undergraduate BME students often have low attitudes towards and knowledge of computer programming, although it is a highly sought-after skill post-graduation [259]. A classroom intervention was developed that combines project-based learning (PBL) and scaffolding through lectures with active learning activities, labs, and an open-ended project. To assess this intervention, we quantified gains in conceptual knowledge via concept maps [220] and attitudes via Likert-type scale survey questions adapted from the attitudes towards computer programming scale (AtCPS) [161], [198]. Reflections from this action research cycle and recommendations for future iterations can be found in section 9.2.

I have also struggled in my education because it is difficult for me to see myself as an engineer based on stereotypical characteristics that are associated with achieving in STEM. Specific aspects of STEM culture lead to many underrepresented groups struggling to develop a sense of identity and belonging with STEM, including work-devotion, apoliticism, technical/social dualism, and stereotypes. Members of the LGBTQIA+ community have been shown to experience a chilly climate in STEM leading to lower retention, closeting, and poor mental health [191], [232]. Critical action research can be used to improve the climate in STEM for queer individuals through developing interventions based on the aspects of culture that lead to marginalization. In Chapter 7, we used critical action research to improve the climate in STEM at the University of Michigan for members of the LGBTQIA+ community with a visibility initiative. Assessment strategies, including to assess sense of belonging and identity with the STEM field, queer community, and university, were implemented to determine the impacts of this initiative, characterize the climate, and provide needs identification for queer individuals in STEM at the university. One full action research cycle was described in Chapter 7, while reflections and implications for future work from the second cycle can be found in section 9.3.

9.2 Improving Computer Programming Education for BME

To apply the action research framework to improve educational strategies used to teach computer programing to BME students, we assessed the implementation of our intervention with surveys and concept maps to demonstrate student gains in conceptual knowledge, perceived gains in skills-based knowledge, perceived applicability of skills, and high perceptions of instructors. Students indicated in the post-module survey that the module increased their knowledge in both image processing and computer programming (above 4.5 on a 5-point Likert-type scale). Significant increases were found from pre- to post-module in confidence with image processing and use of computer programming to solve BME problems. Students' attitudes toward instructor support and PBL had average scores of above 4 on a 5-point Likert-type scale, indicating that students had high perception of instructors and instruction techniques. The significant increase in concept map holistic scores from pre- to post-module time points indicates that students gained conceptual knowledge from the module. Students demonstrated clear gains in skills acquisition and self-efficacy with the material from the module, which suggests that our implementation of research-based pedagogy positively impacted students.

Based on our assessments, future iterations of this module should be adjusted to improve student learning outcomes, engagement, and satisfaction with the module. Some students indicated they were not satisfied with the difficulty of the module in the post-module survey, which could be addressed without increasing the difficulty for those with less prior programming experience by increasing the number and difficulty of lab extra credit portions and by adjusting the difficulty or expectations of the final project. The difficulty of the extra credit portions of labs could be increased by providing more open-ended questions or by providing more explicit correctness criteria for existing questions. The difficulty of the final project script deliverable could be altered by including an explicit requirement that students use methods outside of those covered in the lab portion of the module. Two of five submissions solely used functions that were introduced in labs; including this requirement would have pushed the students in those two groups to further extend their knowledge and promote self-directed learning. Furthermore, extra credit could be given to students who continue to improve and add features to their code after meeting baseline expectations. In the last class periods, the students who had finished their project scripts used class time for other work or finishing of deliverables. In the future, these students should be encouraged to continue innovating on the methods in their final scripts.

Aside from adjusting the requirement for the final script, there are several other potential adjustments to the module that would promote acquisition of self-directed learning skills. To better guide students' self-directed learning, instructors could walk students through an explicit process, such as the problem-based learning cycle described by Hmelo-Silver [260]. In this process, students identify known data related to the problem, generate hypotheses or design ideas to address the problem, identify knowledge gaps, and lastly develop an action plan to cover those knowledge gaps and test their hypotheses or design ideas. When conducting this process, students write out key details on a white board or in a shared document to make the process more concrete. In the current iteration of this module, students conducted the self-directed learning process with ad hoc, unstructured guidance, which may have limited the types of solutions that they posed during the final project.

To promote abstraction from the specific problem to other contexts, more structured reflection opportunities could be given [260]. Concept maps and written reports were the only opportunities for reflection; however, concept maps were not graded, and the written report did not include any criteria related to reflecting on the knowledge acquired during the final project. Grading the concept maps would encourage students to take the assignment more seriously and could provide an opportunity to reflect on how conceptual knowledge grew because of the module [174]. Further, the instruction for how to create concept maps should be expanded to show multiple examples of various complexities, so students are not limited in their understanding of concept map creation. A modified written report rubric could encourage students to explicitly identify the new conceptual and skills-based knowledge that they acquired and explain how this knowledge could be applied to different problems. Both modifications to the current module design would encourage critical reflection and abstraction away from the specific problem presented during the final project.

To specifically aid in the perceived usefulness of the material, which we did not see gains in via our assessment methods, additional connections between the module content and professional practice should be included in future iterations. The panel of experts who use the methods covered in the course that was canceled due to the Covid-19 pandemic may have improved students' perceptions of the usefulness of material as well. An alternative explanation for the lack of statistical significance in perceptions of usefulness of material is that students entered the course with high perceptions of usefulness, which limited the potential for gains in this area. The means for metrics of perceived usefulness were ≥ 4 out of 5 at the first time point, which supports this hypothesis.

As mentioned in chapter 7, future work should also expand this course format for a full 14-week semester, 3-credit class. While the 1-credit course format allowed for students to explore computer programming in a low-stakes, interest-driven environment, a full-semester course would further help students gain computer programming skills and increase the depth of knowledge covered. Additional image process topics could then be added, including filtering methods, artificial intelligence (AI), and 3D segmentation. In longer iterations of the course, we recommend expanding the PBL portions, either through a longer, more open-ended project or by including multiple projects through the course with increasing difficulty. When adapting to a longer course format, implementation of teaching strategies should be assessed frequently, as implications from this action research cycle may not be applicable. With more students enrolled, discussions and teamwork expectations should be explicitly defined to facilitate inclusive and effective communication between students. During labs, students should be encouraged to work together to solve similar problems so that the instructor is able to help struggling students efficiently. The teaching strategies could be adapted for other applications of computer programming and groups of students, but it is important to be cognizant that the implementation and assessment described in this work is for its specific context. The action research framework can be used to help improve the implementation of research-based pedagogy for other contexts in the future.

9.3 Improving the Climate in STEM for Queer Individuals

The visibility initiative developed to improve the climate in STEM for queer individuals was assessed via perceived gains in sense of belonging and identity with their STEM fields, queer community, and university and focus group conversations about the initiative. In Chapter 7, the general discussion of the implications of the assessment results for the climate in STEM for queer individuals was presented. Overall, the response to the STEM Pride initiative is largely positive. Assessment participants perceived positive effects (mean of 5 point Likert-type scale question > 3.25) of STEM Pride on their sense of belonging and identity with their field, queer community, and university and mentioned positive impacts of the initiative, including by reducing feelings of isolation, building queer community, and increasing comfort being out in STEM. Despite the positive effects perceived by individuals, we did not observe impacts on the sense of belonging and identity for the queer population in STEM at the university. Potential reasons for this include the small number of assessment participants who engaged with the STEM Pride initiative and the impacts of other external factors. In this section, we discuss suggested improvements for the next action research cycle for STEM Pride, including adjustments from the direct assessment of the initiative and additional components to incorporate based on needs identification from characterization of climate.

Only a small number of total survey participants (22/112 or 20%) actively engaged with the initiative at the third timepoint, which is likely a large contributing factor for the lack of effect observed in the total survey population. While the number of survey participants who engaged with the initiative increased from timepoint 2 (from 18 to 22), this was not proportional to the increase in survey participants (from 61 to 112), which suggests that efforts to increase engagement with the initiative after the first action research cycle were not successful. To better increase engagement going forward, advertising for the initiative should include an in-person component, through flyers and speaking at new student orientations. We plan to increase the number of events we host and continue to build connections with other identity-based organizations in STEM, including with non-queer focused associations like Society of Women Engineers (SWE), National Society of Black Engineers (NSBE), Society of Hispanic Professional Engineers (SHPE), etc. Despite the small percentage of followers who participated in our assessment, the number of followers on the STEM Pride at UM Instagram page increased between timepoints 2 and 3 (from 200 to 400). It is possible that the assessment survey was not appropriately advertised to Instagram followers or that new followers on the page were not queer individuals in STEM at the University of Michigan. In the future, the survey should be better marketed on the Instagram page, potentially with an added incentive for followers to participate.

Additional aspects will be added to the STEM Pride initiative to allow for participation of queer community members who are not fully out in all aspects of their lives. Based on the limitations of only using Instagram and conversations with those who are not out to everyone in their lives, we will be incorporating an email newsletter in the STEM Pride initiative, which will include the features posted on the Instagram page and event information from STEM Pride and other university or community-based organizations. People who wished to be featured will have the option to be featured on the Instagram, email newsletter, or both, which will hopefully encourage participation from those who do not want everyone they connect with on Instagram to have access to information about their queer identities. We hope to promote opportunities to build community by including information about other organizations' events. Since many of our focus group participants were not able to successfully use university resources to build community, we hope to also provide connections to community-based organizations. Additionally, we will share advice and perspectives from people who are not comfortable sharing their identities from an anonymous form. Since many participants discussed not being fully out via assessment methods, showing this perspective will hopefully decrease levels of isolation and build a sense of belonging in this subset of individuals.

Another topic frequently mentioned during the assessment was the fluidity of identity and participants not wanting to be featured because it would require them to label themselves. To help with this, we plan on sharing reflections from people who have been previously featured about how their queer identities have changed since they were featured. In addition to highlighting the flexibility of identity, this gives us the opportunity to also highlight more STEM achievements and advice from people previously featured. The individual features can also be posted without mention to specific queer identities or with labels like "questioning," which should be more explicit on the Instagram page, recruitment materials, and post information forms. The features themselves could also be adapted to place less importance on individuals' specific queer identities, although this may decrease engagement from and positive effects on individuals with identities that have less representation in the queer community. For example, participants mentioned wanting to increase visibility for asexual identities specifically and these individuals may be less likely to engage if the centrality of specific queer identities is reduced.

Based on general characterization of the climate in STEM, we identified additional needs of the queer community in STEM at our university to incorporate into STEM Pride or future DEI initiatives. Clustering of survey data allowed us to identify demographic factors that contributed to the sense of belonging and identity of participants. Of particular importance, we found that gay men engineering graduate students had a lower sense of belonging and identity with the queer community than other queer women graduate students and queer undergraduate students, which may be connected to hegemonic masculinity in engineering fields. To help address this, the STEM Pride initiative should aim to highlight more gay men on the page and host discussions about perceived masculinity in STEM fields, which will hopefully help expand and encourage critical reflections on the accepted forms of masculinity in STEM spaces. Focus group participants also mentioned feeling more comfortable concerning their queerness in spaces with better gender parity, so efforts should also focus on better acceptance of feminine-presenting individuals in STEM.

In addition to hegemonic masculinity, discourses in STEM culture that enforce cis- and hetero-normative were identified through the analysis of focus groups using queer theory. Specifically, we found that apoliticism, technical/social dualism, overemphasis on objectivity, and expectations of work devotion made participants feel uncomfortable integrating their queer identities in STEM spaces. Incorporating conversations of the identity-based and social aspects of STEM would aid in dismantling these by creating an environment where individuals are comfortable discussing aspects of their identities. Classes should achieve this through acknowledging diverse inventors, examining the impacts of course content on individuals, discussing social justice-based ethical issues, reflecting on the impact of power and privilege on STEM work, and emphasizing the importance communication skills for STEM professionals. If they are comfortable, instructors should share aspects of their personal lives and identities, so that students gain a wholistic view of STEM professionals. STEM students should be taught about qualitative research methods and frameworks to gain perspectives of other research frameworks than positivism. Research labs should connect to the communities that are impacted by their research outputs, discuss the importance of context during analysis of results, and provide wholistic mentoring that encompasses communication, networking, and ethical decision making skills. If they are comfortable, advisors should discuss the struggles they've faced to achieve in STEM with their students. Administrators and departments should acknowledge the impact of wider societal issues on students and faculty and ensure that support resources are widely available.

Specific efforts should be performed to improve the climate in STEM for trans+ individuals. Participants mentioned being frustrated over the lack of discussion of trans+ identities included during conversations about gender minorities in STEM, including in DEI initiatives, paper demographics, and panel discussions. This can be addressed through the education of those who perform DEI work on the proper incorporation of trans+ issues in these conversations and greater awareness of available resources. Similarly, participants also mentioned feeling burdened to educate non-queer individuals about queer issues, including the proper use of pronouns and how to respect trans+ identities. STEM Pride will organize more education-based events, specifically aiming to educate people with power in STEM departments about trans+ issues. These education events should include proper use of pronouns, phrasing of gender-based demographic questions, avoiding microaggressions towards the queer community, and the creation of trans+ inclusive safe spaces. STEM Pride could also have a method for queer individuals to request the education of their departments about a specific queer issue based on their personal experiences. This would allow us to relay information to the department about the impact of the lack of education on students, hopefully increasing buy-in from people in power.

The climate in STEM for queer individuals lead to participants feeling isolated and being unable to build queer community. Participants who engaged with the STEM Pride initiative described its ability to lessen these effects. We have already discussed ways to increase the ability for STEM Pride to further lessen feelings of isolation, through increasing engagement and providing a space for people who are not out. To help more queer individuals in STEM build community, we hope to provide resources in person at new student orientations about queer-focused organizations and host more community building events. We want to plan some events in connection to the non-university centered community, which can help expand the networks students draw from to build their community. Participants also described feeling angry and frustrated about the climate in STEM for queer individuals. We would like to help students cope with these feelings through support groups and providing resources for mental health support.

Based in queer in STEM identity theory, we identified the strategies individuals use to navigate their queer identities in STEM, which included covering based on environmental signals. Specifically, participants discussed the aspects of an environment that signaled safety to share queer identities, including pride flags or stickers, explicit sharing of pronouns, and visibility of other queer individuals. Administrators and educators should be educated about the importance of these signs and encouraged to display these signals to create a comfortable space for queer individuals. Graduate student participants discussed the specific use of these signs when choosing a school or academic advisor. Graduate school recruitment weekends should give queer prospective students opportunities to discuss with current students about the school climate towards queer individuals to aid them in making an informed decision. Participants also mentioned wanting to incorporate queer issues into their STEM research to better combing their queer and STEM identities. Going forward, this project should incorporate aspects of participatory research to provide participants opportunities to be engaged with the study. This will also better aid in improving the climate in STEM, as participants will gain community and be motivated to participate in the resulting DEI efforts.

Chapter 10 Conclusions

10.1 Conclusions

In this thesis, action research was used to improve general practice for hypertension research and STEM education. Technical action research was used to optimize experimental methods to improve the generalizability of results from murine models of hypertension and cardiovascular aging. Practical action research was used to improve implementation of research-based instructional methods for teaching computer programming to biomedical engineering students. Finally, critical action research was used to develop a visibility campaign to improve the climate in STEM for queer individuals. For most of these studies, only one action research cycle was completed and suggestions for future iterations were discussed. Going forward, these studies should be continued, which would result in greater improvements in standards of practice. Despite this limitation, the action research framework proved to be valuable for all of these applications, leading to suggested improvements and increases in general knowledge. A brief summary of each aim is presented below, including contributions and suggested improvements.

10.2 Aim 1: Generalizability of Results for Studying Cardiovascular Aging: Analysis of Naturally Aged Mouse Model.

FSI models of the central vasculature were created for young and old humans and mice at corresponding ages in their lifespans. Because experimental methods were necessarily different between species, strategies were employed to ensure consistency in the use of data to inform computational models. Importantly, a workflow was developed to combine population and clinical data to assign anisotropic stiffness values for human subjects. Key similarities and differences were identified between species and as a result of aging, including in spatially varying patterns of structure and hemodynamics down the aorta. Based on these similarities and differences, suggested improvements to practice include utilizing the C57BL/6 x 129/SvEv naturally aged mouse model for studying specific effects of aging informed by similarities between species, exploring murine models with alterations to elastin structure and differential aortic stiffening, and performing similar comparisons of hemodynamics and structure with age for other mouse strains or animal models.

10.3 Aim 2: Generalizability of Results for Studying Cardiovascular Aging: Impact of Isoflurane Anesthesia with Angiotensin-II Infused Mice

The impact of isoflurane anesthesia on Angiotensin-II (AngII) infused mice was investigated with FSI modeling of the cardiovascular system. A novel computational workflow was developed to estimate awake hemodynamic and vascular structural conditions from anesthetized computational models of the cardiovascular system. We found that isoflurane anesthesia has a larger effect on blood pressure, vascular stiffness, thickness, diameter, and resistance of chronically AngII-infused mice compared to controls. Furthermore, these larger effects were such that trends in pulse pressure and vascular stiffness were reversed in the anesthetized models compared to awake. After accounting for the effects of isoflurane, we were able to analyze the effects of AngII-infusion on *in vivo* hemodynamics and vascular structure. To avoid the differential effects of isoflurane when characterizing hemodynamics of AngII-infused mice, investigators should consider the use of non-volatile anesthetics, explore the minimum required dose for these mice, and potentially adjust for the effects of anesthesia through computational modeling.

10.4 Aim 3: Instructional Practices for Teaching Computer Programming: Applying Research-based Teaching Strategies

Research-based instructional strategies were implemented into a 1-credit, 4-week applied computer programming short course for biomedical engineering students. Project based learning (PBL) and scaffolding were incorporated through lectures with active learning activities, labs, and a final project. Gains in conceptual knowledge and attitudes towards computer programming were assessed at pre-, mid-, and post-course timepoints through concept maps and surveys, respectively. Instructional strategies were found to increase conceptual knowledge and confidence with computer programming and image processing from the pre- to post-course. Student perspectives of the strategies were also high, although some students indicated that they were unhappy with the difficulty and depth of knowledge presented in the course. Suggested improvements for future iterations include expanding to a full length course, increasing the amount and difficulty of lab extra credit questions and projects, showing students a better range of concept maps in terms of complexity before data collection, incorporating more opportunities for reflection, and connecting to BME professional practice.

10.5 Aim 4: Climate in STEM for LGBTQIA+ Individuals: Development of a Visibility Campaign

Based on prior literature concerning the chilly climate in STEM for queer individuals, a visibility initiative, STEM Pride, was developed to highlight the accomplishment, interests, and connections to the LGBTQIA+ community for self-nominating queer individuals in STEM at the University of Michigan (UM). Surveys and focus groups were developed to assess the impact of the initiative, characterize the climate in STEM, and identify needs of the queer community in STEM at three timepoints (fall 2021, winter 2022, and winter 2023). Specifically, surveys quantified sense of belonging and identity with STEM field, queer community, and the university, while focus group discussions concerned experiences related to queer identity in their department, university, and field. No significant differences were identified based on time at any of the three timepoints, although participants generally felt a stronger sense of belonging and identity to the queer community than to their field or university. Although no significant population-based effects were observed, participants who engaged with the initiative described positive effects on decreasing feelings of isolation, building community, and being more comfortable to share queer identities in STEM. Suggested improvements for the future include increasing awareness and engagement with the initiative, perform more education focused events, advocate for inclusion of trans+ specific issues in efforts to increase gender diversity in STEM, expanding the initiative to more than one platform, and highlighting the fluidity of queer identities.

Appendices

Appendix A: IntroCAD Survey Questions

Pre-course survey:

- Open response questions:
  - What is your major?
  - What are your career goals? (it's okay if you don't know yet!!)
  - Why did you enroll in this course?
  - How do you think this course will help you with your career goals?
  - What do you expect from this course?
  - In which classes have you used MATLAB?
  - Have you taken previous computer coding courses? If so, please list them:
  - What is the importance of computer programming for you based on your previous experiences?
- Multiple choice questions:
  - What is your current class standing?
    - First Year
    - Second Year
    - Third Year
    - Fourth Year
    - Graduate Student
    - Other
  - Have you coded in MATLAB before?

        - Yes
        - Maybe
        - No
    - Do you feel confident in your coding ability in MATLAB?
        - Yes
        - Maybe
        - No
    - What is your gender identity?
        - Male
        - Female
        - Other (please specify)
    - Have you participated in a problem-based-learning course previously?
        - Yes
        - Maybe
        - No
- Likert Scale Questions
    - Please rate your confidence (*I've never heard of it; I've heard of it, but I don't know what it is; I know what it is, but not how to use it; I know how to use it*) with the following topics:
        - Variables
        - Vectors
        - Functions
        - Commenting

    - Matrices
    - If-statements
    - For-loops
    - Data Plotting
  - Which languages do you feel confident (*Extremely confident; Somewhat confident; Not confident*) coding in?
    - Python
    - C/C++/C-sharp
    - Java
    - Matlab
    - Other
  - Please rate your agreement (*Strongly disagree; Somewhat disagree; Neither agree nor disagree; Somewhat agree; Strongly* agree) with the following statements:
    - Coding skills are important for biomedical engineers in industry
    - It is important for me to learn coding skills
    - I feel confident in my ability to digitally manipulate medical images
    - I can work with others to write a program
    - I can use computer programming to solve biomedical engineering problems

Mid-Course Survey:

- Open Responses:
  - Approximately how much time are you spending on this course outside of class?
  - What is working well in this course?

        - What would you change about this course going forward?
- Likert Scale Questions:
    - Please rate your agreement (*Strongly disagree; Somewhat disagree; Neither agree nor disagree; Somewhat agree; Strongly* agree) with the following statements:
        - I feel confident in my ability to code in MATLAB
        - I feel confident in my ability to digitally manipulate medical images
        - I can use computer programming to solve biomedical engineering problems

Post-Course Survey:

- Open Responses:
    - When I signed up for the module, I was expecting....
    - Were those expectations met? Why or why not?
    - What I got from the module was....
    - Could you explain how you think this course will be helpful to your current goals or career plans?
    - What would you change about the course?
- Likert Scale Questions:
    - Which languages do you feel confident (*Extremely confident; Somewhat confident; Not confident*) coding in?
        - Python
        - C/C++/C-sharp
        - Java
        - Matlab

    - Other
- Please rate your agreement (*Strongly disagree; Somewhat disagree; Neither agree nor disagree; Somewhat agree; Strongly* agree) with the following statements:
    - Coding skills are important for biomedical engineers in industry
    - It is important for me to learn coding skills
    - I feel confident in my ability to digitally manipulate medical images
    - I can work with others to write a program
    - I can use computer programming to solve biomedical engineering problems
    - Intro to Computer-Aided Diagnosis increased my knowledge of computer programming
    - Intro to Computer-Aided Diagnosis increased my knowledge of image processing
    - Intro to Computer-Aided Diagnosis increased my confidence in computer programming
    - The problem-based-learning techniques used in intro to Computer-Aided Diagnosis increased my learning
- During the module sessions and on other occasions, the graduate student teaching team… (*Never; Sometimes; About half the time; Most of the time; Always)*. Note that the graduate student teaching team were the primary instructors. The graduate student teaching team was observed and mentored by a faculty member.
    - Explained how to solve specific questions
    - Helped me understand key course concepts

- Related the content of the course to a big picture
- Acknowledged my misunderstanding of a concept
- Helped my team when we needed assistance
- Addressed my individual needs or concerns
- Provided responses that guided me in problem solving

Appendix B: STEM Pride Likert-type Scale Survey Questions

Belonging with STEM Field

People in my field share similar values as me

I feel valued by people in my field

I feel excluded by people in my field

I feel like I can be myself in my field

I feel like I belong in my field

Belonging with University

I can really be myself at the university

I feel like I belong at the university

It has been easy for me to make friends at the university

I see myself as a part of the university community

Belonging with LGBTQIA+ Community

I feel like I can be myself in the LGBTQIA+ community

I feel like I belong in the LGBTQIA+ community

I see myself as a part of the LGBTQIA+ community

I feel accepted by people in the LGBTQIA+ community

Identity with STEM Field

Being in my field is an important reflection of who I am

I am happy to be in my field

I fit in well with others in my field

I feel uneasy with others in my field

Identity with University

Being affiliated with the university is an important reflection of who I am

I am happy to be at the university

I identify with others at the university

Identity with LGBTQIA+ Community

Being a member of the LGBTQIA+ community is an important reflection of who I am

I am happy to be a member of the LGBTQIA+ community

I fit in well with others in the LGBTQIA+ community

I feel uneasy with others in the LGBTQIA+ community

Effect of STEM Pride at UM on Belonging in University

STEM Pride at UM has helped me feel like I can be myself more at the university

STEM Pride at UM has helped me feel like I belong more at the university

Effect of STEM Pride at UM on Belonging in Field

STEM Pride at UM has helped me feel more accepted by people in my field

Effect of STEM Pride at UM on Belonging in LGBTQIA+ community in University

STEM Pride at UM has helped me feel like I belong in the LGBTQIA+ community at the university

STEM Pride at UM has helped me feel like I can be myself more within the LGBTQIA community at the university

Effect of STEM Pride at UM on Identity in LGBTQIA+ community in Field

STEM Pride at UM has helped me feel stronger ties to other LGBTQIA+ individuals in my field

Effect of STEM Pride at UM on Identity in Field

STEM Pride at UM has helped me feel prouder to be in my field

Effect of STEM Pride at UM on Identity in LGBTQIA+ community at the University

STEM Pride at UM has helped me feel like I fit in more with the LGBTQIA+ community at UM

Bibliography

[1] S. Kemmis, “What is to be done? The place of action research,” *Educ. Action Res.*, vol. 18, no. 4, pp. 417–427, Dec. 2010, doi: 10.1080/09650792.2010.524745.

[2] S. Kemmis, R. McTaggart, and R. Nixon, “Introducing Critical Participatory Action Research,” in *The Action Research Planner: Doing Critical Participatory Action Research*, S. Kemmis, R. McTaggart, and R. Nixon, Eds., Singapore: Springer, 2014, pp. 1–31. doi: 10.1007/978-981-4560-67-2_1.

[3] J. McNiff, *Action Research: Principles and practice*, 3rd ed. London: Routledge, 2013. doi: 10.4324/9780203112755.

[4] P. Newby, *Research Methods for Education, second edition*. London: Routledge, 2014. doi: 10.4324/9781315758763.

[5] R. McClure, “Common data collection strategies effective in qualitative studies using action research in technical/operational training programs,” *Retrieved May*, Jan. 2002, Accessed: May 02, 2023. [Online]. Available: https://www.academia.edu/718757/Common_data_collection_strategies_effective_in_qualitative_studies_using_action_research_in_technical_operational_training_programs

[6] S. Azhar, I. Ahmad, and M. K. Sein, “Action Research as a Proactive Research Method for Construction Engineering and Management,” *J. Constr. Eng. Manag.*, vol. 136, no. 1, pp. 87–98, Jan. 2010, doi: 10.1061/(ASCE)CO.1943-7862.0000081.

[7] S. Kemmis, “Action research as a practice-based practice,” *Educ. Action Res.*, vol. 17, no. 3, pp. 463–474, Sep. 2009, doi: 10.1080/09650790903093284.

[8] R. Wieringa and A. Moralı, “Technical Action Research as a Validation Method in Information Systems Design Science,” in *Design Science Research in Information Systems. Advances in Theory and Practice*, K. Peffers, M. Rothenberger, and B. Kuechler, Eds., in Lecture Notes in Computer Science. Berlin, Heidelberg: Springer, 2012, pp. 220–238. doi: 10.1007/978-3-642-29863-9_17.

[9] P. Coughlan and D. Coghlan, “Action research for operations management,” *Int. J. Oper. Prod. Manag.*, vol. 22, no. 2, pp. 220–240, Jan. 2002, doi: 10.1108/01443570210417515.

[10] R. J. Wieringa, “Technical Action Research,” in *Design Science Methodology for Information Systems and Software Engineering*, R. J. Wieringa, Ed., Berlin, Heidelberg: Springer, 2014, pp. 269–293. doi: 10.1007/978-3-662-43839-8_19.

[11] F. Burstein and S. Gregor, “The systems development or engineering approach to research in information systems: an action research perspective,” Sep. 2016, doi: 10.4225/03/57DA300758D88.

[12] J. M. Case and G. Light, “Emerging Research Methodologies in Engineering Education Research,” *J. Eng. Educ.*, vol. 100, no. 1, pp. 186–210, 2011, doi: 10.1002/j.2168-9830.2011.tb00008.x.

[13] M. Daniels, Å. Cajander, A. Pears, and T. Clear, “Engineering Education Research in Practice : Evolving use of open ended group projects as a pedagogical strategy for developing skills in global collaboration,” *Int. J. Eng. Educ.*, vol. 26, no. 4, pp. 795–806, 2010.

[14] K. Garg and V. Varma, “A Study of the Effectiveness of Case Study Approach in Software Engineering Education,” in *20th Conference on Software Engineering Education & Training (CSEET’07)*, Jul. 2007, pp. 309–316. doi: 10.1109/CSEET.2007.8.

[15] R. Baskerville and M. D. Myers, “Special Issue on Action Research in Information Systems: Making IS Research Relevant to Practice: Foreword,” *MIS Q.*, vol. 28, no. 3, pp. 329–335, 2004, doi: 10.2307/25148642.

[16] J. A. Mejia, R. A. Revelo, I. Villanueva, and J. Mejia, “Critical Theoretical Frameworks in Engineering Education: An Anti-Deficit and Liberative Approach,” *Educ. Sci.*, vol. 8, no. 4, Art. no. 4, Dec. 2018, doi: 10.3390/educsci8040158.

[17] “Facts About Hypertension,” *CDC*, Jan. 05, 2023.

[18] “Know Your Risk for High Blood Pressure,” *CDC*, Mar. 17, 2023.

[19] “High blood pressure (hypertension) - Causes - NHS.” https://www.nhs.uk/conditions/high-blood-pressure-hypertension/causes/ (accessed Apr. 04, 2023).

[20] “High Blood Pressure Symptoms and Causes | cdc.gov.” https://www.cdc.gov/bloodpressure/about.htm (accessed Apr. 04, 2023).

[21] G. F. Mitchell, “Effects of central arterial aging on the structure and function of the peripheral vasculature: Implications for end-organ damage,” *J. Appl. Physiol.*, vol. 105, no. 5, pp. 1652–1660, 2008, doi: 10.1152/japplphysiol.90549.2008.

[22] J. D. Humphrey, D. G. Harrison, C. A. Figueroa, P. Lacolley, and S. Laurent, “Central Artery stiffness in hypertension and aging a problem with cause and consequence,” *Circ. Res.*, vol. 118, no. 3, pp. 379–381, 2016, doi: 10.1161/circresaha.115.307722.

[23] S. Laurent and P. Boutouyrie, “The Structural Factor of Hypertension,” *Circ. Res.*, vol. 116, no. 6, pp. 1007–1021, Mar. 2015, doi: 10.1161/CIRCRESAHA.116.303596.

[24] R. L. Hamlin and R. A. Altschuld, “Extrapolation from mouse to man,” *Circ. Cardiovasc. Imaging*, vol. 4, no. 1, pp. 2–4, 2011, doi: 10.1161/circimaging.110.961979.

[25] G. F. Mitchell, “Arterial stiffness and wave reflection: Biomarkers of cardiovascular risk,” *Artery Res.*, vol. 3, no. 2, pp. 56–64, 2009, doi: 10.1016/j.artres.2009.02.002.

[26] J. Ferruzzi, M. R. Bersi, and J. D. Humphrey, “Biomechanical phenotyping of central arteries in health and disease: Advantages of and methods for murine models,” *Ann. Biomed. Eng.*, vol. 41, no. 7, pp. 1311–1330, 2013, doi: 10.1007/s10439-013-0799-1.

[27] F. Kum and J. Karalliedde, “Critical appraisal of the differential effects of antihypertensive agents on arterial stiffness,” *Integr. Blood Press. Control*, vol. 3, pp. 63–71, Jun. 2010, doi: 10.2147/IBPC.S6651.

[28] Y. S. Oh *et al.*, “A Special Report on the NHLBI Initiative to Study Cellular and Molecular Mechanisms of Arterial Stiffness and Its Association With Hypertension,” *Circ. Res.*, vol. 121, no. 11, pp. 1216–1218, Nov. 2017, doi: 10.1161/CIRCRESAHA.117.311703.

[29] J. D. (Jay D. Humphrey, “Cardiovascular solid mechanics : cells, tissues, and organs,” p. 757, 2002.

[30] G. A. Holzapfel, “Collagen in arterial walls: Biomechanical aspects,” *Collagen Struct. Mech.*, pp. 285–324, 2008, doi: 10.1007/978-0-387-73906-9_11/COVER.

[31] M. Butlin and A. P. Avolio, “Age-Related Changes in the Mechanical Properties of Large Arteries,” pp. 37–74, 2015, doi: 10.1007/978-3-319-03970-1_3.

[32] J. Ferruzzi, M. R. Bersi, S. Uman, H. Yanagisawa, and J. D. Humphrey, “Decreased Elastic Energy Storage, Not Increased Material Stiffness, Characterizes Central Artery Dysfunction in Fibulin-5 Deficiency Independent of Sex,” *J. Biomech. Eng.*, vol. 137, no. 3, pp. 1–14, 2015, doi: 10.1115/1.4029431.

[33] R. L. Gleason, S. P. Gray, E. Wilson, and J. D. Humphrey, "A multiaxial computer-controlled organ culture and biomechanical device for mouse carotid arteries," *J. Biomech. Eng.*, vol. 126, no. 6, pp. 787–795, 2004, doi: 10.1115/1.1824130.

[34] F. Cuomo, S. Roccabianca, Desmond. Dillon-Murphy, N. Xiao, J. D. Humphrey, and C. Alberto. Figueroa, "Effects of age-associated regional changes in human central artery mechanics on systemic hemodynamics revealed by computational modeling," *PLoS One*, vol. 12, no. 3, pp. 1–21, 2017, doi: 10.7302/Z24B2Z7Z.

[35] S. Roccabianca, C. A. Figueroa, G. Tellides, and J. D. Humphrey, "Quantification of regional differences in aortic stiffness in the aging human," *J. Mech. Behav. Biomed. Mater.*, vol. 29, pp. 618–634, 2014, doi: 10.1016/j.jmbbm.2013.01.026.

[36] J. Xu and G. P. Shi, "Vascular wall extracellular matrix proteins and vascular diseases," *Biochim. Biophys. Acta*, vol. 1842, no. 11, p. 2106, Nov. 2014, doi: 10.1016/J.BBADIS.2014.07.008.

[37] S. M. Arribas, A. Hinek, and M. C. González, "Elastic fibres and vascular structure in hypertension," *Pharmacol. Ther.*, vol. 111, no. 3, pp. 771–791, Sep. 2006, doi: 10.1016/j.pharmthera.2005.12.003.

[38] W. W. Nichols, "Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms," *Am. J. Hypertens.*, vol. 18, no. 1 SUPPL., pp. 3–10, Jan. 2005, doi: 10.1016/J.AMJHYPER.2004.10.009/2/AJH.3S.F5.JPEG.

[39] P. Boutouyrie, P. Chowienczyk, J. D. Humphrey, and G. F. Mitchell, "Arterial Stiffness and Cardiovascular Risk in Hypertension," *Circ. Res.*, vol. 128, pp. 864–886, 2021, doi: 10.1161/circresaha.121.318061.

[40] W. Motz, S. Scheler, B. Schwartzkopff, and B. E. Strauer, "Evaluation of Cardiac Damage in Hypertension:," *http://dx.doi.org/10.1177/174182679500200103*, vol. 2, no. 1, pp. 16–26, Aug. 2016, doi: 10.1177/174182679500200103.

[41] T. Athaya and S. Choi, "A Review of Noninvasive Methodologies to Estimate the Blood Pressure Waveform," *Sensors*, vol. 22, no. 10, May 2022, doi: 10.3390/S22103953.

[42] M. E. Safar, G. E. Plante, and A. Mimran, "Arterial stiffness, pulse pressure, and the kidney," *Am. J. Hypertens.*, vol. 28, no. 5, pp. 561–569, 2015, doi: 10.1093/ajh/hpu206.

[43] M. Butlin and A. Qasem, "Large Artery Stiffness Assessment Using SphygmoCor Technology," *Pulse*, vol. 4, no. 4, pp. 180–192, Jan. 2017, doi: 10.1159/000452448.

[44] M. G. Schultz *et al.*, "Validation Study to Determine the Accuracy of Central Blood Pressure Measurement Using the Sphygmocor Xcel Cuff Device," *Hypertension*, vol. 76, no. 1, pp. 244–250, Jul. 2020, doi: 10.1161/HYPERTENSIONAHA.120.14916.

[45] H. Smulyan and M. E. Safar, "Systolic Blood Pressure Revisited," *J. Am. Coll. Cardiol.*, vol. 29, no. 7, pp. 1407–1413, Jun. 1997, doi: 10.1016/S0735-1097(97)00081-8.

[46] S. Laurent *et al.*, "Expert consensus document on arterial stiffness: Methodological issues and clinical applications," *Eur. Heart J.*, vol. 27, no. 21, pp. 2588–2605, 2006, doi: 10.1093/eurheartj/ehl254.

[47] P. Boutouyrie, M. Briet, C. Collin, S. Vermeersch, and B. Pannier, "Assessment of pulse wave velocity," *Artery Res.*, vol. 3, no. 1, p. 3, 2008, doi: 10.1016/j.artres.2008.11.002.

[48] F. Cuomo *et al.*, "Sex-dependent differences in central artery haemodynamics in normal and fibulin-5 deficient mice: Implications for ageing," *Proc. R. Soc. Math. Phys. Eng. Sci.*, vol. 475, no. 2221, 2019, doi: 10.1098/rspa.2018.0076.

[49] P. Segers *et al.*, “Limitations and pitfalls of non-invasive measurement of arterial pressure wave reflections and pulse wave velocity,” *Artery Res.*, vol. 3, no. 2, pp. 79–88, Jun. 2009, doi: 10.1016/j.artres.2009.02.006.

[50] T. Weber *et al.*, “Noninvasive determination of carotid–femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement,” *J. Hypertens.*, vol. 27, no. 8, pp. 1624–1630, Aug. 2009, doi: 10.1097/HJH.0b013e32832cb04e.

[51] S. Ciurică *et al.*, “Arterial Tortuosity,” *Hypertension*, vol. 73, no. 5, pp. 951–960, May 2019, doi: 10.1161/hypertensionaha.118.11647.

[52] M. H. G. Heusinkveld *et al.*, “Augmentation index is not a proxy for wave reflection magnitude: Mechanistic analysis using a computational model,” *J. Appl. Physiol.*, vol. 127, no. 2, pp. 491–500, 2019, doi: 10.1152/JAPPLPHYSIOL.00769.2018/ASSET/IMAGES/LARGE/ZDG0071930790007.JPEG.

[53] I. B. Wilkinson, H. MacCallum, L. Flint, J. R. Cockcroft, D. E. Newby, and D. J. Webb, “The influence of heart rate on augmentation index and central arterial pressure in humans,” *J. Physiol.*, vol. 525, no. Pt 1, p. 263, May 2000, doi: 10.1111/J.1469-7793.2000.T01-1-00263.X.

[54] F. Fantin, A. Mattocks, C. J. Bulpitt, W. Banya, and C. Rajkumar, “Is augmentation index a good measure of vascular stiffness in the elderly?,” *Age Ageing*, vol. 36, no. 1, pp. 43–48, Jan. 2007, doi: 10.1093/AGEING/AFL115.

[55] E. C. Godia *et al.*, “Carotid Artery Distensibility,” *J. Ultrasound Med. Off. J. Am. Inst. Ultrasound Med.*, vol. 26, no. 9, pp. 1157–1165, Sep. 2007.

[56] L. O. Lerman *et al.*, “Animal Models of Hypertension: A Scientific Statement From the American Heart Association,” *Hypertens. Dallas Tex 1979*, vol. 73, no. 6, pp. e87–e120, Jun. 2019, doi: 10.1161/HYP.0000000000000090.

[57] “Rodent models of hypertension - Jama - 2022 - British Journal of Pharmacology - Wiley Online Library.” https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/bph.15650 (accessed Apr. 04, 2023).

[58] L. Monassier, R. Combe, and L. E. Fertak, “Mouse models of hypertension,” *Drug Discov. Today Dis. Models*, vol. 3, no. 3, pp. 273–281, Sep. 2006, doi: 10.1016/j.ddmod.2006.10.008.

[59] “When are mice considered old?,” *The Jackson Laboratory*. https://www.jax.org/news-and-insights/jax-blog/2017/November/when-are-mice-considered-old (accessed Apr. 04, 2023).

[60] J. S. Colonnello *et al.*, “Transient exposure to elastase induces mouse aortic wall smooth muscle cell production of MCP-1 and RANTES during development of experimental aortic aneurysm,” *J. Vasc. Surg.*, vol. 38, no. 1, pp. 138–146, Jul. 2003, doi: 10.1016/S0741-5214(03)00125-3.

[61] H. Yanagisawa *et al.*, “Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo,” *Nature*, vol. 415, no. 6868, pp. 168–171, 2002, doi: 10.1038/415168a.

[62] C. F. Deschepper, J. L. Olson, M. Otis, and N. Gallo-Payet, “Characterization of blood pressure and morphological traits in cardiovascular-related organs in 13 different inbred mouse strains,” *J. Appl. Physiol.*, vol. 97, no. 1, pp. 369–376, Jul. 2004, doi: 10.1152/japplphysiol.00073.2004.

[63] G. D. Fink, "Does Tail-Cuff Plethysmography Provide a Reliable Estimate of Central Blood Pressure in Mice?," *J. Am. Heart Assoc.*, vol. 6, no. 6, Nov. 2017, doi: 10.1161/JAHA.117.006554.

[64] K. L. Navarro, M. Huss, J. C. Smith, P. Sharp, J. O. Marx, and C. Pacharinsak, "Mouse Anesthesia: The Art and Science," *ILAR J.*, vol. 62, no. 1–2, pp. 238–273, Dec. 2021, doi: 10.1093/ILAR/ILAB016.

[65] N. Nama, M. Aguirre, J. D. Humphrey, and C. A. Figueroa, "A nonlinear rotation-free shell formulation with prestressing for vascular biomechanics," *Sci. Rep.*, vol. 10, no. 1, pp. 1–17, 2020, doi: 10.1038/s41598-020-74277-5.

[66] C. A. Figueroa, I. E. Vignon-Clementel, K. E. Jansen, T. J. R. Hughes, and C. A. Taylor, "A coupled momentum method for modeling blood flow in three-dimensional deformable arteries," *Comput. Methods Appl. Mech. Eng.*, vol. 195, no. 41–43, pp. 5685–5706, 2006, doi: 10.1016/j.cma.2005.11.011.

[67] C. J. Arthurs *et al.*, "CRIMSON: An open-source software framework for cardiovascular integrated modelling and simulation," *PLOS Comput. Biol.*, vol. 17, no. 5, p. e1008881, May 2021, doi: 10.1371/journal.pcbi.1008881.

[68] I. E. Vignon-Clementel, A. Figueroa, K. E. Jansen, and C. A. Taylor, "Outflow boundary conditions for three-dimensional finite element modeling of blood flow and pressure in arteries," *Comput Methods Appl Mech Engrg*, vol. 195, pp. 3776–3796, 2006, doi: 10.1016/j.cma.2005.04.014.

[69] K. D. Lau and · C Alberto Figueroa, "Simulation of short-term pressure regulation during the tilt test in a coupled 3D-0D closed-loop model of the circulation," *Biomech Model Mechanobiol*, doi: 10.1007/s10237-014-0645-x.

[70] C. J. Arthurs, K. D. Lau, K. N. Asrress, S. R. Redwood, and C. Alberto Figueroa, "A mathematical model of coronary blood flow control: Simulation of patientspecific three-dimensional hemodynamics during exercise," *Am. J. Physiol. - Heart Circ. Physiol.*, vol. 310, no. 9, pp. H1242–H1258, May 2016, doi: 10.1152/ajpheart.00517.2015.

[71] S. Baek, R. L. Gleason, K. R. Rajagopal, and J. D. Humphrey, "Theory of small on large: Potential utility in computations of fluid-solid interactions in arteries," *Comput. Methods Appl. Mech. Eng.*, vol. 196, no. 31–32, pp. 3070–3078, 2007, doi: 10.1016/j.cma.2006.06.018.

[72] J. Ferruzzi, P. D. Achille, G. Tellides, and J. D. Humphrey, "Combining in vivo and in vitro biomechanical data reveals key roles of perivascular tethering in central artery function," *PLoS ONE*, vol. 13, no. 9, pp. 1–21, 2018, doi: 10.1371/journal.pone.0201379.

[73] P. Moireau *et al.*, "External tissue support and fluid-structure simulation in blood flows," *Biomech. Model. Mechanobiol.*, vol. 11, pp. 1–18, 2012, doi: 10.1007/s10237-011-0289-z.

[74] J. Ferruzzi, D. A. Vorp, and J. D. Humphrey, "On constitutive descriptors of the biaxial mechanical behaviour of human abdominal aorta and aneurysms," *J. R. Soc. Interface*, vol. 8, no. 56, pp. 435–450, 2011, doi: 10.1098/rsif.2010.0299.

[75] J. Alastruey, N. Xiao, H. Fok, T. Schaeffter, and C. A. Figueroa, "On the impact of modelling assumptions in multi-scale, subject-specific models of aortic haemodynamics," *J. R. Soc. Interface*, vol. 13, no. 119, 2016, doi: 10.1098/rsif.2016.0073.

[76] A. C. Simon *et al.*, "An evaluation of large arteries compliance in man," 1979.

[77] E. G. Lakatta, M. Wang, and S. S. Najjar, "Arterial Aging and Subclinical Arterial Disease are Fundamentally Intertwined at Macroscopic and Molecular Levels," *Med. Clin. North Am.*, vol. 93, no. 3, pp. 583–604, 2009, doi: 10.1016/j.mcna.2009.02.008.

[78] M. E. Safar, "Arterial aging-hemodynamic changes and therapeutic options," *Nat. Rev. Cardiol.*, vol. 7, no. 8, pp. 442–449, 2010, doi: 10.1038/nrcardio.2010.96.

[79] S. Greenwald, "Ageing of the conduit arteries," *J. Pathol.*, vol. 211, no. 2, pp. 157–172, Jan. 2007, doi: 10.1002/path.2101.

[80] P. Doevendans, "Cardiovascular phenotyping in mice," *Cardiovasc. Res.*, vol. 39, no. 1, pp. 34–49, Jul. 1998, doi: 10.1016/S0008-6363(98)00073-X.

[81] M. Brandfonbrener, M. Landowne, and N. W. Shock, "Changes in Cardiac Output with Age," *Circulation*, vol. 12, no. 4, pp. 557–566, Oct. 1955, doi: 10.1161/01.cir.12.4.557.

[82] D. Papadimitriou, T. Xanthos, I. Dontas, P. Lelovas, and D. Perrea, "The use of mice and rats as animal models for cardiopulmonary resuscitation research," *Lab. Anim.*, vol. 42, no. 3, pp. 265–276, 2008, doi: 10.1258/la.2007.006035.

[83] E. C. Davis, "Stability of elastin in the developing mouse aorta: a quantitative radioautographic study," *Histochemistry*, vol. 100, no. 1, pp. 17–26, 1993, doi: 10.1007/BF00268874.

[84] B. S. Fleenor, K. D. Marshall, J. R. Durrant, L. A. Lesniewski, and D. R. Seals, "Arterial stiffening with ageing is associated with transforming growth factor-β1-related changes in adventitial collagen: Reversal by aerobic exercise," *J. Physiol.*, vol. 588, no. 20, pp. 3971–3982, 2010, doi: 10.1113/jphysiol.2010.194753.

[85] J. Ferruzzi, D. Madziva, A. W. Caulk, G. Tellides, and J. D. Humphrey, "Compromised mechanical homeostasis in arterial aging and associated cardiovascular consequences," *Biomech. Model. Mechanobiol.*, vol. 17, no. 5, pp. 1281–1295, 2018, doi: 10.1007/s10237-018-1026-7.

[86] H. Yanagisawa and J. Wagenseil, "Elastic fibers and biomechanics of the aorta: Insights from mouse studies," *Matrix Biol.*, vol. 85–86, pp. 160–172, 2020, doi: 10.1016/j.matbio.2019.03.001.

[87] R. L. Gleason, S. P. Gray, E. Wilson, and J. D. Humphrey, "A multiaxial computer-controlled organ culture and biomechanical device for mouse carotid arteries," *J. Biomech. Eng.*, vol. 126, no. 6, pp. 787–795, 2004, doi: 10.1115/1.1824130.

[88] J. T. Lee *et al.*, "Design and validation of Segment - freely available software for cardiovascular image analysis," *BMC Med. Imaging*, vol. 8, no. 1, p. 10, 2008, doi: 10.1186/1471-2342-8-10.

[89] F. Cuomo, J. Ferruzzi, J. D. Humphrey, and C. A. Figueroa, "An Experimental–Computational Study of Catheter Induced Alterations in Pulse Wave Velocity in Anesthetized Mice," *Ann. Biomed. Eng.*, vol. 43, no. 7, pp. 1555–1570, 2015, doi: 10.1007/s10439-015-1272-0.

[90] N. Xiao, J. Alastruey, and C. Alberto Figueroa, "A systematic comparison between 1-D and 3-D hemodynamics in compliant arterial models," *Int. J. Numer. Methods Biomed. Eng.*, vol. 30, no. 2, pp. 204–231, Feb. 2014, doi: 10.1002/cnm.2598.

[91] A. Redheuil *et al.*, "Reduced ascending aortic strain and distensibility: Earliest manifestations of vascular aging in humans," *Hypertension*, vol. 55, no. 2, pp. 319–326, 2010, doi: 10.1161/hypertensionaha.109.141275.

[92] N. R. Gaddum, J. Alastruey, P. Beerbaum, P. Chowienczyk, and T. Schaeffter, "A technical assessment of pulse wave velocity algorithms applied to non-invasive arterial waveforms," *Ann. Biomed. Eng.*, vol. 41, no. 12, pp. 2617–2629, 2013, doi: 10.1007/s10439-013-0854-y.

[93] R. Katori, "Normal Cardiac Output in Relation to Age and Body Size," *Tohoku J. Exp. Med.*, vol. 128, no. 4, pp. 377–387, 1979, doi: 10.1620/tjem.128.377.

[94] J. Hashimoto and S. Ito, “Aortic blood flow reversal determines renal function: Potential explanation for renal dysfunction caused by aortic stiffening in hypertension,” *Hypertension*, vol. 66, no. 1, pp. 61–67, Jul. 2015, doi: 10.1161/HYPERTENSIONAHA.115.05236.

[95] D. Craiem *et al.*, “Aging impact on thoracic aorta 3D morphometry in intermediate-risk subjects: Looking beyond coronary arteries with non-contrast cardiac CT,” *Ann. Biomed. Eng.*, vol. 40, no. 5, pp. 1028–1038, 2012, doi: 10.1007/s10439-011-0487-y.

[96] B. Rylski, B. Desjardins, W. Moser, J. E. Bavaria, and R. K. Milewski, “Gender-related changes in aortic geometry throughout life,” *Eur. J. Cardiothorac. Surg.*, vol. 45, no. 5, pp. 805–811, 2014, doi: 10.1093/ejcts/ezt597.

[97] M. R. Bersi, R. Khosravi, A. J. Wujciak, D. G. Harrison, and J. D. Humphrey, “Differential cell-matrix mechanoadaptations and inflammation drive regional propensities to aortic fibrosis, aneurysm or dissection in hypertension,” *J. R. Soc. Interface*, vol. 14, no. 136, 2017, doi: 10.1098/rsif.2017.0327.

[98] M. E. Safar and P. Laurent, “Pulse pressure and arterial stiffness in rats: Comparison with humans,” *Am. J. Physiol. - Heart Circ. Physiol.*, vol. 285, no. 4 54-4, pp. 1363–1369, 2003, doi: 10.1152/ajpheart.00513.2003.

[99] M. F. O’Rourke and J. Hashimoto, “Mechanical Factors in Arterial Aging. A Clinical Perspective,” *J. Am. Coll. Cardiol.*, vol. 50, no. 1, pp. 1–13, 2007, doi: 10.1016/j.jacc.2006.12.050.

[100] F. U. S. Mattace-Raso *et al.*, “Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: ‘Establishing normal and reference values,’” *Eur. Heart J.*, vol. 31, no. 19, pp. 2338–2350, 2010, doi: 10.1093/eurheartj/ehq165.

[101] N. Saouti, N. Westerhof, P. E. Postmus, and A. Vonk-Noordegraaf, “The arterial load in pulmonary hypertension,” *Eur. Respir. Rev.*, vol. 19, no. 117, pp. 197–203, Sep. 2010, doi: 10.1183/09059180.00002210.

[102] M. E. Safar, V. Regnault, and P. Lacolley, “Sex Differences in Arterial Stiffening and Central Pulse Pressure: Mechanistic Insights?,” *J. Am. Coll. Cardiol.*, vol. 75, no. 8, pp. 881–883, Mar. 2020, doi: 10.1016/j.jacc.2019.12.041.

[103] V. R. Souza, E. Mendes, M. Casaro, A. T. F. B. Antiorio, F. A. Oliveira, and C. M. Ferreira, “Description of Ovariectomy Protocol in Mice,” *Methods Mol. Biol. Clifton NJ*, vol. 1916, pp. 303–309, 2019, doi: 10.1007/978-1-4939-8994-2_29.

[104] B. Spronck *et al.*, “Excessive adventitial stress drives inflammation-mediated fibrosis in hypertensive aortic remodelling in mice,” *J. R. Soc. Interface*, vol. 18, no. 180, p. 20210336, Jul. 2021, doi: 10.1098/RSIF.2021.0336.

[105] S.-I. Murtada *et al.*, “Paradoxical aortic stiffening and subsequent cardiac dysfunction in Hutchinson–Gilford progeria syndrome,” *J. R. Soc. Interface*, vol. 17, no. 166, p. 20200066, May 2020, doi: 10.1098/rsif.2020.0066.

[106] B. Spronck, J. Ferruzzi, C. Bellini, A. W. Caulk, S.-I. Murtada, and J. D. Humphrey, “Aortic remodeling is modest and sex-independent in mice when hypertension is superimposed on aging,” *J. Hypertens.*, vol. 38, no. 7, pp. 1312–1321, Jul. 2020, doi: 10.1097/HJH.0000000000002400.

[107] W. C. Seyde and D. E. Longnecker, “Anesthetic influences on regional hemodynamics in normal and hemorrhaged rats.,” *Anesthesiology*, vol. 61, no. 6, pp. 686–698, Dec. 1984, doi: 10.1097/00000542-198412000-00010.

[108] S. Jung *et al.*, “Isoflurane inhibition of endocytosis is an anesthetic mechanism of action,” *Curr. Biol.*, vol. 32, no. 14, pp. 3016-3032.e3, Jul. 2022, doi: 10.1016/J.CUB.2022.05.037.

[109] L. A. Low, L. C. Bauer, and B. A. Klaunberg, “Comparing the Effects of Isoflurane and Alpha Chloralose upon Mouse Physiology,” *PLOS ONE*, vol. 11, no. 5, p. e0154936, May 2016, doi: 10.1371/JOURNAL.PONE.0154936.

[110] C. Constantinides and K. Murphy, “Molecular and Integrative Physiological Effects of Isoflurane Anesthesia: The Paradigm of Cardiovascular Studies in Rodents using Magnetic Resonance Imaging,” *Front. Cardiovasc. Med.*, vol. 3, p. 23, Jul. 2016, doi: 10.3389/FCVM.2016.00023/BIBTEX.

[111] M. A. Sparks, S. D. Crowley, S. B. Gurley, M. Mirotsou, and T. M. Coffman, “Classical Renin-Angiotensin system in kidney physiology,” *Compr. Physiol.*, vol. 4, no. 3, pp. 1201–1228, 2014, doi: 10.1002/CPHY.C130040.

[112] C. M. Ferrario, J. L. VonCannon, K. N. Wright, and S. Ahmad, “Chapter 11 - Renin–angiotensin–aldosterone system inhibitors. New and old approaches,” in *Angiotensin*, P. M. Pilowsky, Ed., in Molecular Mediators in Health and Disease: How Cells Communicate. Academic Press, 2023, pp. 317–334. doi: 10.1016/B978-0-323-99618-1.00013-1.

[113] A. R. Lucarini, E. Picano, and A. Salvetti, “Coronary microvascular disease in hypertensives,” *Clin. Exp. Hypertens.*, vol. A14, no. 1–2, pp. 55–66, 1992, doi: 10.3109/10641969209036171.

[114] P. M. Pilowsky, Ed., “Front Matter,” in *Angiotensin*, in Molecular Mediators in Health and Disease: How Cells Communicate. Academic Press, 2023, p. iii. doi: 10.1016/B978-0-323-99618-1.01001-1.

[115] A. C. Simões-e-Silva, P. A. S. Vaz de Castro, L. Bitencourt, J. L. de Oliveira Campos, S. B. C. Soares de Brito, and B. L. Fischer, “Chapter 3 - Renin–angiotensin system and inflammation,” in *Angiotensin*, P. M. Pilowsky, Ed., in Molecular Mediators in Health and Disease: How Cells Communicate. Academic Press, 2023, pp. 77–105. doi: 10.1016/B978-0-323-99618-1.00003-9.

[116] J. Ullman, R. Härgestam, S. Lindahl, S. H. H. Chan, S. Eriksson, and M. Rundgren, “Circulatory effects of angiotensin II during anaesthesia, evaluated by real-time spectral analysis,” *Acta Anaesthesiol. Scand.*, vol. 47, no. 5, pp. 532–540, May 2003, doi: 10.1034/J.1399-6576.2003.00114.X.

[117] E. Samain *et al.*, “Isoflurane Alters Angiotensin II–Induced Ca2+Mobilization in Aortic Smooth Muscle Cells from Hypertensive RatsImplication of Cytoskeleton,” *Anesthesiology*, vol. 97, no. 3, pp. 642–651, Sep. 2002, doi: 10.1097/00000542-200209000-00019.

[118] J. Yu, K. Ogawa, Y. Tokinaga, S. Iwahashi, and Y. Hatano, “The vascular relaxing effects of sevoflurane and isoflurane are more important in hypertensive than in normotensive rats,” *Can. J. Anesth. 2004 5110*, vol. 51, no. 10, pp. 979–985, Apr. 2014, doi: 10.1007/BF03018483.

[119] A. Ishikawa, K. Ogawa, Y. Tokinaga, N. Uematsu, K. Mizumoto, and Y. Hatano, “The mechanism behind the inhibitory effect of isoflurane on angiotensin II-induced vascular contraction is different from that of sevoflurane,” *Anesth. Analg.*, vol. 105, no. 1, pp. 97–102, Jul. 2007, doi: 10.1213/01.ANE.0000265851.37923.EC.

[120] S. E. Hopper *et al.*, “Comparative Study of Human and Murine Aortic Biomechanics and Hemodynamics in Vascular Aging,” *Front. Physiol.*, vol. 12, p. 1889, Oct. 2021, doi: 10.3389/FPHYS.2021.746796/BIBTEX.

[121] J. S. Swaney, "Impact of anesthesia on cardiac function during echocardiography in mice," *Am J Physiol Heart Circ Physiol*, vol. 282, no. 6, 2002, doi: 10.1152/ajpheart.00845.2001.

[122] S. Takuma *et al.*, "Anesthetic inhibition in ischemic and nonischemic murine heart: Comparison with conscious echocardiographic approach," *Am. J. Physiol. - Heart Circ. Physiol.*, vol. 280, no. 5 49-5, 2001, doi: 10.1152/AJPHEART.2001.280.5.H2364/ASSET/IMAGES/LARGE/H40510763003.JPEG.

[123] T. P. Tan *et al.*, "Assessment of cardiac function by echocardiography in conscious and anesthetized mice: importance of the autonomic nervous system and disease state," *J. Cardiovasc. Pharmacol.*, vol. 42, no. 2, pp. 182–190, Aug. 2003, doi: 10.1097/00005344-200308000-00005.

[124] J. D. Humphrey, "Mechanisms of Arterial Remodeling in Hypertension," *Hypertension*, vol. 52, no. 2, pp. 195–200, Aug. 2008, doi: 10.1161/HYPERTENSIONAHA.107.103440.

[125] S.-I. Murtada, Y. Kawamura, D. Weiss, and J. D. Humphrey, "Differential biomechanical responses of elastic and muscular arteries to angiotensin II-induced hypertension," *J. Biomech.*, vol. 119, p. 110297, Apr. 2021, doi: 10.1016/j.jbiomech.2021.110297.

[126] M. E. Charlson, C. R. MacKenzie, J. P. Gold, K. L. Ales, M. Topkins, and G. T. Shires, "Preoperative characteristics predicting intraoperative hypotension and hypertension among hypertensives and diabetics undergoing noncardiac surgery.," *Ann. Surg.*, vol. 212, no. 1, pp. 66–81, Jul. 1990.

[127] P. Hoppe *et al.*, "Chronic arterial hypertension and nocturnal non-dipping predict postinduction and intraoperative hypotension: A secondary analysis of a prospective study," *J. Clin. Anesth.*, vol. 79, p. 110715, Aug. 2022, doi: 10.1016/j.jclinane.2022.110715.

[128] S. J. Howell, J. W. Sear, and P. Foëx, "Hypertension, hypertensive heart disease and perioperative cardiac risk†," *BJA Br. J. Anaesth.*, vol. 92, no. 4, pp. 570–583, Apr. 2004, doi: 10.1093/bja/aeh091.

[129] A. S. Alhakak, J. R. Teerlink, J. Lindenfeld, M. Böhm, G. M. C. Rosano, and T. Biering-Sørensen, "The significance of left ventricular ejection time in heart failure with reduced ejection fraction," *Eur. J. Heart Fail.*, vol. 23, pp. 541–551, 2021, doi: 10.1002/ejhf.2125.

[130] Z. Xu, H. Okamoto, M. Akino, H. Onozuka, Y. Matsui, and H. Tsutsui, "Pravastatin attenuates left ventricular remodeling and diastolic dysfunction in angiotensin II-induced hypertensive mice," *J. Cardiovasc. Pharmacol.*, vol. 51, no. 1, pp. 62–70, 2008, doi: 10.1097/FJC.0b013e31815bb629.

[131] N. A. Noll, H. Lal, and W. D. Merryman, "Mouse Models of Heart Failure with Preserved or Reduced Ejection Fraction," *Am. J. Pathol.*, vol. 190, no. 8, pp. 1596–1608, Aug. 2020, doi: 10.1016/j.ajpath.2020.04.006.

[132] C. Withaar, C. S. P. Lam, G. G. Schiattarella, R. A. de Boer, and L. M. G. Meems, "Heart failure with preserved ejection fraction in humans and mice: embracing clinical complexity in mouse models," *Eur. Heart J.*, vol. 42, no. 43, pp. 4420–4430, Nov. 2021, doi: 10.1093/EURHEARTJ/EHAB389.

[133] M. Valero-Muñoz, W. Backman, and F. Sam, "Murine Models of Heart Failure With Preserved Ejection Fraction: A 'Fishing Expedition,'" *JACC Basic Transl. Sci.*, vol. 2, no. 6, pp. 770–789, Dec. 2017, doi: 10.1016/j.jacbts.2017.07.013.

[134] S. Horgan, C. Watson, N. Glezeva, and J. Baugh, "Murine models of diastolic dysfunction and heart failure with preserved ejection fraction," *J. Card. Fail.*, vol. 20, no. 12, pp. 984–995, Dec. 2014, doi: 10.1016/j.cardfail.2014.09.001.

[135] R. Pourmodheji *et al.*, “Computational modelling of multi-temporal ventricular–vascular interactions during the progression of pulmonary arterial hypertension,” *J. R. Soc. Interface*, vol. 19, no. 196, p. 20220534, Nov. 2022, doi: 10.1098/rsif.2022.0534.

[136] A. Korneva and J. D. Humphrey, “Maladaptive aortic remodeling in hypertension associates with dysfunctional smooth muscle contractility,” *https://doi.org/10.1152/ajpheart.00503.2017*, vol. 316, no. 2, pp. H265–H278, Feb. 2019, doi: 10.1152/AJPHEART.00503.2017.

[137] E. Wilde *et al.*, “Tail-cuff technique and its influence on central blood pressure in the mouse,” *J. Am. Heart Assoc.*, vol. 6, no. 6, Nov. 2017, doi: 10.1161/JAHA.116.005204.

[138] X. Zhao, D. Ho, S. Gao, C. Hong, D. E. Vatner, and S. F. Vatner, “Arterial Pressure Monitoring in Mice,” *Curr. Protoc. Mouse Biol.*, vol. 1, p. 105, Mar. 2011, doi: 10.1002/9780470942390.MO100149.

[139] D. Weiss, A. S. Long, G. Tellides, S. Avril, J. D. Humphrey, and M. R. Bersi, “Evolving Mural Defects, Dilatation, and Biomechanical Dysfunction in Angiotensin II–Induced Thoracic Aortopathies,” *Arterioscler. Thromb. Vasc. Biol.*, vol. 42, no. 8, pp. 973–986, 2022.

[140] F. Qi, K. Ogawa, Y. Tokinaga, N. Uematsu, T. Minonishi, and Y. Hatano, “Volatile anesthetics inhibit angiotensin II-induced vascular contraction by modulating myosin light chain phosphatase inhibiting protein, CPI-17 and regulatory subunit, MYPT1 phosphorylation,” *Anesth. Analg.*, vol. 109, no. 2, pp. 412–417, 2009, doi: 10.1213/ANE.0B013E3181AC6D96.

[141] R. K. Ream, J. L. Lewis, B. Echeverria, and R. N. Page, “Trust Matters: Distinction and Diversity in Undergraduate Science Education,” *Teach. Coll. Rec. Voice Scholarsh. Educ.*, vol. 116, no. 4, pp. 1–50, Apr. 2014, doi: 10.1177/016146811411600408.

[142] J. Aspelin, “How do relationships influence student achievement? Understanding student performance from a general, social psychological standpoint,” *Int. Stud. Sociol. Educ.*, vol. 22, no. 1, pp. 41–56, Mar. 2012, doi: 10.1080/09620214.2012.680327.

[143] T. R. Morton and E. C. Parsons, “#BlackGirlMagic: The identity conceptualization of Black women in undergraduate STEM education,” *Sci. Educ.*, vol. 102, no. 6, pp. 1363–1393, 2018, doi: 10.1002/sce.21477.

[144] G. Hackett, N. E. Betz, J. M. Casas, and I. A. Rocha-Singh, “Gender, ethnicity, and social cognitive factors predicting the academic achievement of students in engineering,” *J. Couns. Psychol.*, vol. 39, pp. 527–538, 1992, doi: 10.1037/0022-0167.39.4.527.

[145] T. L. Strayhorn, *Student Development Theory in Higher Education: A Social Psychological Approach*. New York: Routledge, 2015. doi: 10.4324/9780203458211.

[146] D. Davis-Reid, *UNDERSTANDING HELP-SEEKING INTENTION AND AWARENESS OF MARITAL*. 2019. doi: 10.13140/RG.2.2.11565.64486.

[147] D. H. Schunk and M. K. DiBenedetto, “Motivation and social cognitive theory,” *Contemp. Educ. Psychol.*, vol. 60, p. 101832, Jan. 2020, doi: 10.1016/j.cedpsych.2019.101832.

[148] C. Good, A. Rattan, and C. S. Dweck, “Why do women opt out? Sense of belonging and women’s representation in mathematics,” *J. Pers. Soc. Psychol.*, vol. 102, no. 4, pp. 700–717, Apr. 2012, doi: 10.1037/A0026659.

[149] A. M. Rainey, J. M. Smith, and D. Verdín, “Classroom Practices that Support Minoritized Engineering Students’ Sense of Belonging (Research),” Jul. 2021, doi: 10.18260/1-2--36797.

[150] A. Konak, S. Kulturel-Konak, G. E. O. Kremer, and I. E. Esparragoza, “Teamwork attitude, interest, and self-efficacy: Their implications for teaching teamwork skills to engineering students,” *Proc. - Front. Educ. Conf. FIE*, vol. 2015, Dec. 2015, doi: 10.1109/FIE.2015.7344118.

[151] N. A. Mamaril, E. L. Usher, C. R. Li, D. R. Economy, and M. S. Kennedy, “Measuring Undergraduate Students’ Engineering Self-Efficacy: A Validation Study,” *J. Eng. Educ.*, vol. 105, no. 2, pp. 366–395, 2016, doi: 10.1002/jee.20121.

[152] B. J. Zimmerman, “Self-Efficacy: An Essential Motive to Learn,” *Contemp. Educ. Psychol.*, vol. 25, no. 1, pp. 82–91, Jan. 2000, doi: 10.1006/ceps.1999.1016.

[153] B. A. Al-Sheeb, A. M. Hamouda, and G. M. Abdella, “Modeling of student academic achievement in engineering education using cognitive and non-cognitive factors,” *J. Appl. Res. High. Educ.*, vol. 11, no. 2, pp. 178–198, Jan. 2019, doi: 10.1108/JARHE-10-2017-0120.

[154] A. Carpi, D. M. Ronan, H. M. Falconer, and N. H. Lents, “Cultivating minority scientists: Undergraduate research increases self-efficacy and career ambitions for underrepresented students in STEM,” *J. Res. Sci. Teach.*, vol. 54, no. 2, pp. 169–194, 2017, doi: 10.1002/tea.21341.

[155] T. Luo, W. W. M. So, Z. H. Wan, and W. C. Li, “STEM stereotypes predict students’ STEM career interest via self-efficacy and outcome expectations,” *Int. J. STEM Educ.*, vol. 8, no. 1, p. 36, May 2021, doi: 10.1186/s40594-021-00295-y.

[156] G. Pleiss, M. Perry, and Y. V. Zastavker, “Student self-efficacy in introductory Project-Based Learning courses,” *Proc. - Front. Educ. Conf. FIE*, pp. 8–13, 2012, doi: 10.1109/FIE.2012.6462457.

[157] R. J. Schlegel, S. L. Chu, K. Chen, E. Deuermeyer, A. G. Christy, and F. Quek, “Making in the classroom: Longitudinal evidence of increases in self-efficacy and STEM possible selves over time,” *Comput. Educ.*, vol. 142, p. 103637, Dec. 2019, doi: 10.1016/j.compedu.2019.103637.

[158] M. Syed *et al.*, “The Role of Self-Efficacy and Identity in Mediating the Effects of STEM Support Experiences,” *Anal. Soc. Issues Public Policy*, vol. 19, no. 1, pp. 7–49, 2019, doi: 10.1111/asap.12170.

[159] T. Huziak-Clark, T. Sondergeld, M. van Staaden, C. Knaggs, and A. Bullerjahn, “Assessing the Impact of a Research-Based STEM Program on STEM Majors’ Attitudes and Beliefs,” *Sch. Sci. Math.*, vol. 115, no. 5, pp. 226–236, 2015, doi: 10.1111/ssm.12118.

[160] V. Amnouychokanant, S. Boonlue, S. Chuathong, and K. Thamwipat, “A Study of First-Year Students’ Attitudes toward Programming in the Innovation in Educational Technology Course,” *Educ. Res. Int.*, vol. 2021, p. e9105342, Oct. 2021, doi: 10.1155/2021/9105342.

[161] M. Baser, “Attitude, gender and achievement in computer programming,” *Middle East J. Sci. Res.*, vol. 14, no. 2, pp. 248–255, 2013, doi: 10.5829/idosi.mejsr.2013.14.2.2007.

[162] T. L. Strayhorn, *College Students’ Sense of Belonging: A Key to Educational Success for All Students*. London, UNITED KINGDOM: Routledge, 2012. Accessed: May 16, 2023. [Online]. Available: http://ebookcentral.proquest.com/lib/umichigan/detail.action?docID=1101369

[163] S. Solanki, P. McPartlan, D. Xu, and B. K. Sato, “Success with EASE: Who benefits from a STEM learning community?,” *PLOS ONE*, vol. 14, no. 3, p. e0213827, Mar. 2019, doi: 10.1371/journal.pone.0213827.

[164] R. Dou, Z. Hazari, K. Dabney, G. Sonnert, and P. Sadler, “Early informal STEM experiences and STEM identity: The importance of talking science,” *Sci. Educ.*, vol. 103, no. 3, pp. 623–637, 2019, doi: 10.1002/sce.21499.

[165] R. Dou and H. Cian, “Constructing STEM identity: An expanded structural model for STEM identity research,” *J. Res. Sci. Teach.*, vol. 59, no. 3, pp. 458–490, 2022, doi: 10.1002/tea.21734.

[166] J. R. Morelock, “A systematic literature review of engineering identity: definitions, factors, and interventions affecting development, and means of measurement,” *Eur. J. Eng. Educ.*, vol. 42, no. 6, pp. 1240–1262, Nov. 2017, doi: 10.1080/03043797.2017.1287664.

[167] T. R. Harris, J. D. Bransford, and S. P. Brophy, “Roles for learning sciences and learning technologies in biomedical engineering education: a review of recent advances,” *Annu. Rev. Biomed. Eng.*, vol. 4, pp. 29–48, 2002, doi: 10.1146/annurev.bioeng.4.091701.125502.

[168] *Read “How People Learn: Brain, Mind, Experience, and School: Expanded Edition” at NAP.edu*. doi: 10.17226/9853.

[169] “From Teaching to Learning — A New Paradigm For Undergraduate Education.” https://www.tandfonline.com/doi/epdf/10.1080/00091383.1995.10544672?needAccess=true&role=button (accessed Apr. 27, 2023).

[170] K. A. Smith, S. D. Sheppard, D. W. Johnson, and R. T. Johnson, “Pedagogies of Engagement: Classroom-Based Practices,” *J. Eng. Educ.*, vol. 94, no. 1, pp. 87–101, 2005, doi: 10.1002/j.2168-9830.2005.tb00831.x.

[171] R. M. Felder and R. Brent, *Teaching and Learning STEM: A Practical Guide | Wiley*. Wiley-Blackwell Publishing Ltd, 2016. Accessed: Jun. 01, 2021. [Online]. Available: https://www.wiley.com/en-us/Teaching+and+Learning+STEM%3A+A+Practical+Guide-p-9781118925812

[172] S. Freeman *et al.*, “Active learning increases student performance in science, engineering, and mathematics,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 111, no. 23, pp. 8410–8415, Jun. 2014, doi: 10.1073/pnas.1319030111.

[173] M. Stains *et al.*, “Anatomy of STEM teaching in North American universities,” *Science*, vol. 359, no. 6383, pp. 1468–1470, Mar. 2018, doi: 10.1126/science.aap8892.

[174] W. C. Newstetter, “Fostering Integrative Problem Solving in Biomedical Engineering: The PBL Approach,” *Ann. Biomed. Eng.*, vol. 34, no. 2, pp. 217–225, 2006, doi: 10.1007/s10439-005-9034-z.

[175] T. A. Litzinger *et al.*, “Engineering education and the development of expertise,” *J. Eng. Educ.*, vol. 100, no. 1, pp. 123–150, Jan. 2011, doi: 10.1002/j.2168-9830.2011.tb00006.x.

[176] R. M. Felder and R. Brent, “Cooperative learning,” *ACS Symp. Ser.*, vol. 970, pp. 34–53, 2007, doi: 10.1021/BK-2007-0970.CH004.

[177] C.-M. Hsiung, “The Effectiveness of Cooperative Learning,” *J. Eng. Educ.*, vol. 101, no. 1, pp. 119–137, 2012.

[178] K. Kricorian, M. Seu, D. Lopez, E. Ureta, and O. Equils, “Factors influencing participation of underrepresented students in STEM fields: matched mentors and mindsets,” *Int. J. STEM Educ.*, vol. 7, no. 1, p. 16, Apr. 2020, doi: 10.1186/s40594-020-00219-2.

[179] T. B. Lane, “Beyond Academic and Social Integration: Understanding the Impact of a STEM Enrichment Program on the Retention and Degree Attainment of Underrepresented Students,” *CBE—Life Sci. Educ.*, vol. 15, no. 3, p. ar39, Sep. 2016, doi: 10.1187/cbe.16-01-0070.

[180] D. Ghazzawi, D. Pattison, and C. Horn, "Persistence of Underrepresented Minorities in STEM Fields: Are Summer Bridge Programs Sufficient?," *Front. Educ.*, vol. 6, 2021, Accessed: May 16, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/feduc.2021.630529

[181] H. E. Metcalf, "Disrupting the Pipeline: Critical Analyses of Student Pathways Through Postsecondary STEM Education," *New Dir. Institutional Res.*, vol. 2013, no. 158, pp. 77–93, 2014, doi: 10.1002/ir.20047.

[182] Sophie L. Kuchynka, Asia Eaton, and Luis M. Rivera, "Understanding and Addressing Gender-Based Inequities in STEM: Research Synthesis and Recommendations for U.S. K-12 Education", doi: 10.1111/sipr.12087.

[183] H. B. Carlone and A. Johnson, "Understanding the science experiences of successful women of color: Science identity as an analytic lens," *J. Res. Sci. Teach.*, vol. 44, no. 8, pp. 1187–1218, 2007, doi: 10.1002/tea.20237.

[184] B. E. Rincón and C. E. George-Jackson, "Examining Department Climate for Women in Engineering: The Role of STEM Interventions," *J. Coll. Stud. Dev.*, vol. 57, no. 6, pp. 742–747, 2016, doi: 10.1353/csd.2016.0072.

[185] R. A. Miller and M. Downey, "Examining the STEM Climate for Queer Students with Disabilities," *J. Postsecond. Educ. Disabil.*, vol. 33, no. 2, pp. 169–181, 2020.

[186] M. Ong, J. M. Smith, and L. T. Ko, "Counterspaces for women of color in STEM higher education: Marginal and central spaces for persistence and success," *J. Res. Sci. Teach.*, vol. 55, no. 2, pp. 206–245, 2018, doi: 10.1002/tea.21417.

[187] A. H. Master and A. N. Meltzoff, "Cultural Stereotypes and Sense of Belonging Contribute to Gender Gaps in STEM," 2020. Accessed: May 16, 2023. [Online]. Available: https://eric.ed.gov/?id=ED605235

[188] "Career–life balance for women of color: Experiences in science and engineering academia." https://psycnet.apa.org/doiLanding?doi=10.1037%2Fa0039068 (accessed May 17, 2023).

[189] H. B. Carlone and A. Johnson, "Understanding the science experiences of successful women of color: Science identity as an analytic lens," *J. Res. Sci. Teach.*, vol. 44, no. 8, pp. 1187–1218, 2007, doi: 10.1002/tea.20237.

[190] Y. Tibbetts, J. M. Harackiewicz, S. J. Priniski, and E. A. Canning, "Broadening Participation in the Life Sciences with Social–Psychological Interventions," *CBE—Life Sci. Educ.*, vol. 15, no. 3, p. es4, Sep. 2016, doi: 10.1187/cbe.16-01-0001.

[191] M. Jennings, R. Roscoe, N. Kellam, and S. Jayasuriya, "A Review of the State of LGBTQIA+ Student Research in STEM and Engineering Education," *ASEE Annu. Conf.*, 2020.

[192] "Women of Color in STEM: Navigating the Workforce." https://web.s.ebscohost.com/ehost/ebookviewer/ebook?sid=d916f1be-acaa-4946-af1b-84beeb3a8a32%40redis&ppid=pp_43&vid=0&format=EB (accessed May 17, 2023).

[193] B. J. Casad and W. J. Bryant, "Addressing Stereotype Threat is Critical to Diversity and Inclusion in Organizational Psychology," *Front. Psychol.*, vol. 7, 2016, Accessed: May 17, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fpsyg.2016.00008

[194] M. Borrego, E. P. Douglas, and C. T. Amelink, "Quantitative, Qualitative, and Mixed Research Methods in Engineering Education," *J. Eng. Educ.*, vol. 98, no. 1, pp. 53–66, 2009, doi: 10.1002/j.2168-9830.2009.tb01005.x.

[195] S. B. Merriam and E. J. Tisdell, *Qualitative Research: A Guide to Design and Implementation*. Newark, UNITED STATES: John Wiley & Sons, Incorporated, 2015. Accessed: Apr. 30, 2023. [Online]. Available: http://ebookcentral.proquest.com/lib/umichigan/detail.action?docID=2089475

[196] S. M. Krishnan, “Academic program models for undergraduate biomedical engineering,” in *2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBC 2014*, Institute of Electrical and Electronics Engineers Inc., Nov. 2014, pp. 5145–5148. doi: 10.1109/EMBC.2014.6944783.

[197] J. A. White *et al.*, “Core Competencies for Undergraduates in Bioengineering and Biomedical Engineering: Findings, Consequences, and Recommendations,” *Ann. Biomed. Eng.*, vol. 48, no. 3, pp. 905–912, Mar. 2020, doi: 10.1007/s10439-020-02468-2.

[198] Y. B. İ. R. Çalişma, “A study for determining computer programming students’ attitudes towards programming and their programming self-efficacy / Bilgisayar programcılığı öğrencilerinin programlamaya karşı tutum ve programlama öz-yeterliklerinin belirlenmesine yönelik bir çalı,” *Eğitimde Kuram Ve Uygul.*, vol. 11, no. 1, pp. 51–67, 2014, doi: 10.17244/eku.53204.

[199] E. N. Wiebe, L. Williams, K. Yang, and C. Miller, “Computer Science Attitude Survey,” *Comput. Sci.*, no. January 2003, p. 5, 2003, doi: 10.1024/0301-1526.32.1.54.

[200] H. Shoaib, A. Madamanchi, E. Pienaar, D. M. Umulis, and M. E. Cardella, “‘I Think I Am Getting There’ Understanding the Computational Identity of Engineering Students Participating in a Computationally Intensive Thermodynamics Course,” *Biomed. Eng. Educ.*, vol. 3, no. 1, pp. 1–21, Jan. 2023, doi: 10.1007/s43683-022-00084-1.

[201] T. Marshall, “Biomedical Engineering Education Study: Developing Computer Science Population,” WPI, 2018.

[202] A. Kolmos and E. De Graaff, “Problem-Based and Project-Based Learning in Engineering Education Merging Models,” 2017, doi: 10.1017/CBO9781139013451.012.

[203] J. E. Mills and D. F. Treagust, “Engineering education - is problem based or project based learning the answer?,” *Australas. Assoc. Eng. Educ. Inc ©*, no. June, pp. 1–16, 2014.

[204] M. Savin-Baden 1960-, C. Howell. Major, S. for R. into H. Education., Inc. ebrary, M. Savin-Baden, and C. H. Major, *Foundations of problem-based learning*. in SRHE and Open University Press imprint. Maidenhead, 2004.

[205] A. Shekar, “Project based Learning in Engineering Design Education : Sharing Best Practices Project-Based Learning in Engineering Design Education : Sharing Best Practices,” *ASEE Annu. Conf. Proc.*, 2014.

[206] R. M. Capraro and S. W. Slough, “STEM PBL - Integrated Science Tech Eng Math Approach,” in *STEM Project-Based Learning: An Integrated Science, Technology, Engineering, and Mathematics (STEM) Approach*, R. M. Capraro, M. M. Capraro, and J. Morgan, Eds., Rotterdam, The Netherlands: Sense Publishers, 2013, pp. 1–5.

[207] D. Bédard, C. Lison, D. Dalle, D. Côté, and N. Boutin, “Problem-based and Project-based Learning in Engineering and Medicine: Determinants of Students’ Engagement and Persistance,” *Interdiscip. J. Probl.-Based Learn.*, vol. 6, no. 2, p. 8, Aug. 2012, doi: 10.7771/1541-5015.1355.

[208] M. Frank, I. Lavy, and D. Elata, “Implementing the project-based learning approach in an academic engineering course,” *Int. J. Technol. Des. Educ.*, vol. 13, no. 3, pp. 273–288, 2003, doi: 10.1023/A:1026192113732.

[209] K. Gavin, “Case study of a project-based learning course in civil engineering design,” *Eur. J. Eng. Educ.*, vol. 36, no. 6, pp. 547–558, 2011, doi: 10.1080/03043797.2011.624173.

[210] D. Wood, J. S. Bruner, and G. Ross, “THE ROLE OF TUTORING IN PROBLEM SOLVING,” *J. Child Psychol. Psychiatry*, vol. 17, no. 2, pp. 89–100, Apr. 1976, doi: 10.1111/j.1469-7610.1976.tb00381.x.

[211] J. van de Pol, M. Volman, and J. Beishuizen, “Scaffolding in teacher-student interaction: A decade of research,” *Educ. Psychol. Rev.*, vol. 22, no. 3, pp. 271–296, 2010, doi: 10.1007/s10648-010-9127-6.

[212] R. Mayer, B. Moeller, V. Kaliwata, B. Zweber, R. Stone, and M. Frank, “Educating engineering undergraduates: Effects of scaffolding in a problem-based learning environment,” *Proc. Hum. Factors Ergon. Soc.*, pp. 2507–2511, 2012, doi: 10.1177/1071181312561510.

[213] A. Huang-Saad and E. Springer, “Transforming biomedical engineering education through instructional design,” *Int. J. Eng. Educ.*, vol. 36, no. 3, pp. 865–877, 2020.

[214] J. Strobel and A. van Barneveld, “When is PBL More Effective? A Meta-synthesis of Meta-analyses Comparing PBL to Conventional Classrooms,” *Interdiscip. J. Probl.-Based Learn.*, vol. 3, no. 1, p. 4, Mar. 2009, doi: 10.7771/1541-5015.1046.

[215] A. Carberry, S. Krause, C. Ankeny, and C. Waters, ““Unmuddying” course content using muddiest point reflections,” in *2013 IEEE Frontiers in Education Conference (FIE)*, IEEE, Oct. 2013, pp. 937–942. doi: 10.1109/FIE.2013.6684966.

[216] R. J. Stanley, S. E. Watkins, and R. H. Moss, “Integration of real-world problems into an image processing curriculum,” *Int. J. Eng. Educ.*, vol. 21, no. 2 PART 1, pp. 318–326, 2005.

[217] E. Von Glasersfeld, “Cognition , Construction of Knowledge , and Teaching Author (s): Ernst Von Glasersfeld Source : Synthese , Vol . 80 , No . 1 , History , Philosophy , and Science Teaching (Jul ., 1989), pp . Published by : Springer Stable URL : http://www.jstor.org/st,” *Synthese*, vol. 80, no. 1, pp. 121–140, 2017.

[218] J. D. Novak and A. J. Cañas, “Theoretical Origins of Concept Maps, How to Construct Them, and Uses in Education,” *Reflecting Educ.*, vol. 3, no. 1, pp. 29–42, 2007.

[219] J. D. Novak and D. B. Gowin, *Learning How to Learn*. New York, NY: Cambridge University Press, 1984.

[220] M. E. Besterfield-Sacre, J. Gerchak, M. Lyons, L. J. Shuman, and H. Wolfe, “Scoring Concept Maps : An Integrated,” *J. Eng. Educ.*, no. April, pp. 105–115, 2004, doi: 10.1002/j.2168-9830.2004.tb00795.x.

[221] J. Turns, C. J. Atman, and R. Adams, “Concept maps for engineering education: A cognitively motivated tool supporting varied assessment functions,” *IEEE Trans. Educ.*, vol. 43, no. 2, pp. 164–173, 2000, doi: 10.1109/13.848069.

[222] C. Bodnar, T. R. Christiani, K. Dahm, and A. J. Vernengo, “Implementation and assessment of an undergraduate tissue engineering laboratory course,” *Educ. Chem. Eng.*, vol. 24, pp. 52–59, 2018, doi: 10.1016/j.ece.2018.07.002.

[223] G. W. Ellis, A. Rudnitsky, and B. Silverstein, “Using concept maps to enhance understanding in engineering education,” *Int. J. Eng. Educ.*, vol. 20, no. 6, pp. 1012–1021, 2004.

[224] G. Martínez, Á. L. Pérez, M. I. Suero, and P. J. Pardo, “The Effectiveness of Concept Maps in Teaching Physics Concepts Applied to Engineering Education: Experimental

Comparison of the Amount of Learning Achieved With and Without Concept Maps," *J. Sci. Educ. Technol.*, vol. 22, no. 2, pp. 204–214, 2013, doi: 10.1007/s10956-012-9386-8.

[225] J. Segalàs, D. Ferrer-Balas, and K. F. Mulder, "What do engineering students learn in sustainability courses? The effect of the pedagogical approach," *J. Clean. Prod.*, vol. 18, no. 3, pp. 275–284, 2010, doi: 10.1016/j.jclepro.2009.09.012.

[226] G. Tokdemir and N. E. Cagiltay, "A Concept Map Appraoch for Introduction to Computer Engineering Course Curriculum," in *IEEE Global Engineering Education Conference*, Madrid, Spain, 2010, pp. 243–250.

[227] J. M. T. Walker and P. H. King, "Concept mapping as a form of student assessment and instruction," *ASEE Annu. Conf. Proc.*, no. April, pp. 7151–7166, 2002.

[228] B. E. Hughes, "Coming out in STEM: Factors affecting retention of sexual minority STEM students," *Sci. Adv.*, vol. 4, no. 3, pp. eaao6373–eaao6373, Mar. 2018, doi: 10.1126/sciadv.aao6373.

[229] K. Legg, A. Cofino, and R. Sanlo, "Lesbian, Gay, Bisexual, and Transgender College Students: Revisiting Retention:," *https://doi.org/10.1177/1521025119895513*, vol. 21, no. 4, pp. 417–430, Jan. 2020, doi: 10.1177/1521025119895513.

[230] A. Haverkamp, Ava Butler, Naya Selene Pelzl, Michelle Kay Bothwell, Devlin Montfort, and Quo-Li Diskill, "Exploring Transgender and Gender Nonconforming Engineering Undergrad-uate Experiences through Autoethnography," *ASEE Annu. Conf. Proc.*, 2019.

[231] E. Alberts and N. Rohrsetzer, "Identity and Social Support: LGBTQIA+ Individuals and Help-Seeking Behaviors," *SUURJ Seattle Univ. Undergrad. Res. J.*, vol. 4, p. 11, 2020.

[232] E. A. Cech and W. R. Rothwell, "LGBTQ Inequality in Engineering Education," *J. Eng. Educ.*, vol. 107, no. 4, pp. 583–610, Oct. 2018, doi: 10.1002/jee.20239.

[233] M. A. Haverkamp, "The Complexity of Nonbinary Gender Inclusion in Engineering Culture," *ASEE Annu. Conf. Proc.*, 2018.

[234] J. L. Linley, K. A. Renn, and M. R. Woodford, "EXAMINING THE ECOLOGICAL SYSTEMS OF LGBTQ STEM MAJORS," *J. Women Minor. Sci. Eng.*, vol. 24, no. 1, pp. 1–16, 2018, doi: 10.1615/JWOMENMINORSCIENENG.2017018836.

[235] A. Sona, J. Laboy Santana, and E. K. H. Saitta, "Looking through a Prism: A Systematic Review of LGBTQ+ STEM Literature," *J. Chem. Educ.*, vol. 100, no. 1, pp. 125–133, Jan. 2023, doi: 10.1021/acs.jchemed.2c00391.

[236] K. B. Nash Catherine J., Ed., *Queer Methods and Methodologies: Intersecting Queer Theories and Social Science Research*. London: Routledge, 2016. doi: 10.4324/9781315603223.

[237] W. Faulkner, "'Nuts and bolts and people': Gender-troubled engineering identities," *Soc. Stud. Sci.*, vol. 37, no. 3, pp. 331–356, Jun. 2007, doi: 10.1177/0306312706072175.

[238] E. A. Cech, "The veiling of queerness: Depoliticization and the experiences of LGBT engineers," *ASEE Annu. Conf. Expo. Conf. Proc.*, 2013, doi: 10.18260/1-2--22628.

[239] E. A. Cech and T. J. Waidzunas, "Systemic inequalities for LGBTQ professionals in STEM," *Sci. Adv.*, vol. 7, no. 3, pp. 933–948, Jan. 2021, doi: 10.1126/sciadv.abe0933.

[240] B. E. Hughes, "'Managing by Not Managing': How Gay Engineering Students Manage Sexual Orientation Identity," *J. Coll. Stud. Dev.*, vol. 58, no. 3, pp. 385–401, 2017, doi: 10.1353/csd.2017.0029.

[241] E. A. Cech and T. J. Waidzunas, "Navigating the heteronormativity of engineering: The experiences of lesbian, gay, and bisexual students," *Eng. Stud.*, vol. 3, no. 1, pp. 1–24, Apr. 2011, doi: 10.1080/19378629.2010.545065.

[242] A. Duran, “Queer and of color: A systematic literature review on queer students of color in higher education scholarship,” *J. Divers. High. Educ.*, vol. 12, pp. 390–400, 2019, doi: 10.1037/dhe0000084.

[243] A. Mattheis, D. C.-R. De Arellano, and J. B. Yoder, “A Model of Queer STEM Identity in the Workplace,” *J. Homosex.*, vol. 67, no. 13, pp. 1839–1863, Nov. 2020, doi: 10.1080/00918369.2019.1610632.

[244] “Visibility matters: A conversation with the co-founder of 500 Queer Scientists.” https://www.science.org/content/article/visibility-matters-conversation-co-founder-500-queer-scientists (accessed Jun. 01, 2023).

[245] “Factorial and Criterion Validity of Scores of a Measure of Belonging in Youth Development Programs - Dawn Anderson-Butcher, David E. Conroy, 2002.” https://journals.sagepub.com/doi/10.1177/001316402236882 (accessed Jun. 01, 2023).

[246] D. Chachra, D. Kilgore, H. Loshbaugh, J. Mccain, and H. Chen, “Being and Becoming: Gender and Identity Formation of Engineering Students”, Accessed: Nov. 03, 2021. [Online]. Available: www.engr.washington.edu/caee

[247] J. W. Lounsbury and D. DeNeui, “Collegiate psychological sense of community in relation to size of college/university and extroversion,” *J. Community Psychol.*, vol. 24, pp. 381–394, 1996, doi: 10.1002/(SICI)1520-6629(199610)24:4<381::AID-JCOP7>3.0.CO;2-X.

[248] A. Kassambara and F. Mundt, *Factoextra: Extract and Visualize the Results of Multivariate Data Analyses*. 2020. [Online]. Available: https://CRAN.R-project.org/package=factoextra

[249] J. C. Gower, “A General Coefficient of Similarity and Some of Its Properties,” *Biometrics*, vol. 27, no. 4, pp. 857–871, 1971, doi: 10.2307/2528823.

[250] M. Maechler, P. Rousseeuw, A. Struyf, M. Hubert, and K. Hornik, *cluster: Cluster Analysis Basics and Extensions*. 2022. [Online]. Available: https://CRAN.R-project.org/package=cluster

[251] E. Schubert and P. J. Rousseeuw, “Faster k-Medoids Clustering: Improving the PAM, CLARA, and CLARANS Algorithms,” in *Similarity Search and Applications*, G. Amato, C. Gennaro, V. Oria, and M. Radovanović, Eds., in Lecture Notes in Computer Science. Cham: Springer International Publishing, 2019, pp. 171–187. doi: 10.1007/978-3-030-32047-8_16.

[252] leonard kaufman and Peter J Rousseeuw, *Finding Groups in Data | Wiley Series in Probability and Statistics*. in Wiley Series in Probably and Statistic. John Wiley & Sons, Incorporated, 1990. Accessed: May 25, 2023. [Online]. Available: https://onlinelibrary.wiley.com/doi/book/10.1002/9780470316801

[253] F. Batool and C. Hennig, “Clustering with the Average Silhouette Width,” *Comput. Stat. Data Anal.*, vol. 158, p. 107190, Jun. 2021, doi: 10.1016/j.csda.2021.107190.

[254] J. Han, M. Kamber, and J. Pei, Eds., “Front Matter,” in *Data Mining (Third Edition)*, in The Morgan Kaufmann Series in Data Management Systems. Boston: Morgan Kaufmann, 2012, pp. i–v. doi: 10.1016/B978-0-12-381479-1.00016-2.

[255] “AAPI LGBTQ Students,” *GLSEN*. https://www.glsen.org/research/aapi-lgbtq-students (accessed Jun. 01, 2023).

[256] K. F. Trenshaw, A. Hetrick, R. F. Oswald, S. L. Vostral, and M. C. Loui, “Lesbian, gay, bisexual, and transgender students in engineering: Climate and perceptions,” *Proc. - Front. Educ. Conf. FIE*, pp. 1238–1240, 2013, doi: 10.1109/FIE.2013.6685028.

[257] P. C. Meidlinger and D. A. Hope, “Differentiating disclosure and concealment in measurement of outness for sexual minorities: The Nebraska Outness Scale.,” *Psychol. Sex. Orientat. Gend. Divers.*, vol. 1, no. 4, pp. 489–497, Dec. 2014, doi: 10.1037/sgd0000080.

[258] G. Lopez and N. Chims, “Classroom Concerns of Gay and Lesbian Students: The Invisible Minority,” *Coll. Teach.*, vol. 41, no. 3, pp. 97–103, Jul. 1993, doi: 10.1080/87567555.1993.9926788.

[259] J. A. White *et al.*, “Core Competencies for Undergraduates in Bioengineering and Biomedical Engineering: Findings, Consequences, and Recommendations,” *Ann. Biomed. Eng.*, vol. 48, no. 3, pp. 905–912, 2020, doi: 10.1007/s10439-020-02468-2.

[260] C. E. Hmelo-Silver, “Problem-based learning: What and how do students learn?,” *Educ. Psychol. Rev.*, vol. 16, no. 3, pp. 235–266, Sep. 2004, doi: 10.1023/B:EDPR.0000034022.16470.f3.