Investigating the Link Between Aging-Related Vascular Dysfunction and Peripheral Neuropathy Through Pressure Myography

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INVESTIGATING THE LINK BETWEEN AGING-RELATED VASCULAR DYSFUNCTION AND PERIPHERAL NEUROPATHY THROUGH PRESSURE MYOGRAPHY

by

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Peripheral neuropathy can be defined as any condition that reduces the innervation of peripheral tissues. Pathologies such as diabetes and aging are catalysts for the development of peripheral neuropathy starting in the skin. It is known that neuropathy with aging and obesity extends into adipose in the C57BL/6 mouse model. Losing proper nerve communication between brain and adipose tissue is detrimental to metabolic health because it reduces the capacity of adipose to undergo necessary processes such as browning and lipolysis. Additionally, it can also worsen obesity and diabetes. Since aging increases the risk for these pathologies, a better understanding of neuropathy with aging is necessary to mitigate them. Denervation is observed around vasculature in subcutaneous white adipose tissue (scWAT) with aging, which is thought to contribute to microcirculation dysfunction and reduced cardiometabolic health. To test the integrity of scWAT vasculature with aging, a pressure myography system is being built to apply to scWAT blood vessels – the first time this has been performed in these tissues. Myography measures myogenic tone and vascular resistance, which may be affected by aging or neuropathy. Functional responses and vascular reactivity of an isolated artery from mouse WAT will be tested to quantify vascular function. As part of this project, a custom pressure myography system has been built and tested. Now that the system is fully assembled and capable of sustaining a physiological environment for experimentation, assessments across aging will commence.
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INTRODUCTION

Energy Balance and Adipose Anatomy

The central nervous system (CNS) plays an integral role in human health, including in the maintenance of metabolic homeostasis. Energy input occurs through food consumption and nutrient absorption, and energy expenditure results from bodily movement and metabolic demand [1]. The body must maintain homeostatic regulation of energy intake and expenditure to facilitate numerous bodily processes. These processes are regulated in large part by the hypothalamus in the brain [2]. Neural networks are used throughout the body to send and receive signals to facilitate this delicate balance.

Adipose tissue is especially important for metabolism, as it consists of either white (energy storing) or brown (energy expending) depots.

White adipose tissue (WAT) differs from brown adipose tissue (BAT) in many ways. White adipocytes are generally large, spherical, and contain only one large lipid droplet which comprises the majority of the adipocyte’s volume. They are white in color because this large white lipid droplet pushes all other organelles to the periphery [3]. Contrastingly, brown adipocytes get their color because their organelles contain many smaller lipid droplets and iron-containing mitochondria [3]. These mitochondria contain uncoupling protein 1 (UCP-1), which is a proton transporter that generates heat in place of ATP during non-shivering thermogenesis [3]. There also exists beige and pink adipocyte hues. Beige adipocytes present characteristics of both BAT and WAT and are scattered within WAT instead of being clustered in specific depots [3]. They arise from precursors of WAT and have protective qualities that prevent obesity and metabolic
dysfunction due to their resemblance to BAT [3,4]. Pink adipocytes exist within mammary tissue to assist with milk production [3].

Adipocytes are able to efficiently revert back and forth between lipolysis (the breakdown of fats to release fatty acids and glycerol for energy mobilization) and lipogenesis (the conversion of fatty acids back into triglycerides for energy storage) due to the high innervation and vascularization of the adipose tissue [9]. This functionality is a major proponent to the ability of adipocytes to respond to metabolic regulation.

When this balance is disrupted, it alters the composition and functionality of adipose tissue. Adipose is plastic in nature, as it is capable of hyperplasia (the proliferation of adipocytes) and hypertrophy (the enlargement of adipocytes) [10]. The expansion of adipocytes by hyperplasia is associated with insulin insensitivity and reduced metabolic control, while expansion by hypertrophy is associated with reduced triacylglycerol storage capacity, ectopic lipid deposition and adipocyte necrosis [11]. As the size or number of these cells changes due to metabolic dysregulation, the vascular and neural networks surrounding them are also implicated. This causes further dysfunction and tissue damage. Chronic energy imbalances may manifest in the development of pathologies such as obesity, diabetes, cardiovascular disease, tissue inflammation, adipocyte hypertrophy, kidney disease, stroke, osteoarthritis, malnutrition, and atrophy [12, 13].

There are many adipose depots throughout the body, which are largely shared in terms of anatomical location between mice and humans [5]. The major adipose tissue depots include the visceral, subcutaneous, bone marrow and perivascular compartments [3]. Perivascular adipose tissue (PVAT) surrounds most vasculature and has varying
compositions of BAT and WAT, depending on its location [6]. Thought to be a differentiated continuation of the vascular adventitia (the outermost layer of the blood vessel), PVAT normally functions to release adipokines, chemokines and growth factors [6,7]. However, in metabolically unhealthy individuals, the protective actions of PVAT are diminished [8].

**Innervation of Adipose Tissue**

Adipose tissue communicates with the CNS through the use of afferent sensory and efferent sympathetic nerves [14, 15, 16]. These nerve fibers directly interact with adipocytes in the parenchyma of WAT [17] and BAT [18] in order to release various neurotransmitters. Neurotransmitters are chemical messengers used by the nervous system to propagate messages from the CNS to specific locations in the body. Neurotransmitters are released by neuronal axon terminals into a synaptic cleft before being taken up by the corresponding receptors on the post-synaptic dendrite [19]. One important neurotransmitter is norepinephrine (NE). This monoamine is the primary neurotransmitter in the sympathetic nervous system, as it regulates lipolysis [20], adipogenesis [21], browning [22], and heat loss through peripheral vasoconstriction [23]. The presence of NE within sympathetic innervation in adipose can be experimentally determined through local denervation studies using 6-hydroxydopamine [24] and guanethidine [25]. The release of NE is an essential process, as it allows for the body to respond to metabolically stressful situations. NE is also a known catecholamine, which causes it to act hormonally as well, through its release by the adrenal medulla [26].

Surgical denervation of BAT causes reduced sympathetic control, which results in reduced thermogenesis, a “whitening” of the tissue, and increased fat mass [14, 27]. It
also results in decreased levels of tyrosine hydroxylase, which is involved in catecholamine synthesis [28], and UCP-1, which is involved in thermogenesis [3]. Contrastingly, the surgical denervation of WAT results in increased fat pad mass and white adipocyte proliferation and differentiation [29].

Chemical denervation studies in adipose can also be used to support the presence of sensory nerves by tracing nerve projections in inguinal scWAT (i-scWAT) back to thoracic and lumbar dorsal root ganglia [14, 15]. Although the function of sensory nerves in adipose is not clearly understood, it is possible that they communicate information to the brain about lipid reserves, as well as temperature changes in inguinal BAT [30]. Dysregulation in this circuitry results in increased fat pad mass via hypertrophy and hyperplasia in WAT [31, 32].

**Neurovascular Communication in Adipose**

White adipose tissue has been shown to be highly vascularized due to its high demand for oxygen, nutrients, and immune cell trafficking [33]. This is because adipose tissue growth and adipocyte differentiation rely on the development of new vessels to support increased metabolic demand [34]. Like most tissues, adipose tissue is supplied with arterial blood before venous blood is returned back to the heart [35]. At least one capillary or blood vessel is in close contact with each adipocyte [35]. I-scWAT has been found to have better blood supply than other subcutaneous spaces [36]. This is due to the numerous splitting locations of the feeding vasculature. Unfortunately, no research is available that identifies the specific vessels within the i-scWAT. This is an important step in order to determine the mechanisms behind metabolism-related vascular dysfunction.
The interactions between nerves and vasculature are vast and important, as they permit both local and remote control of peripheral circulation. This is a symbiotic relationship, as they rely on each other for survival and growth. Nerves rely on vasculature because they are damaged in hypoxic conditions, while most vasculature rely on nerves in order to constrict/dilate in response to stimuli. However, vascular tissues such as capillaries are not innervated, and are likely regulated by paracrine signaling and through circulating factors rather than neurotransmitters [37].

When adipocytes do not receive enough oxygen from surrounding capillaries due to hypertrophy, they release cytokines and hormones to regulate a process called angiogenesis, or the formation of new blood vessels. Hypoxia driven angiogenesis is a locally regulated process that promotes the formation of microvasculature such as capillaries [37]. This process starts with the release of endothelial growth factor (VEGFa), which brings surrounding blood vessels closer to exposed nerves [38]. Adipokines such as leptin will also promote angiogenesis through a tyrosine-dependent pathway, while adiponectin will inhibit it through a Rho kinase pathway [39, 40].

Both local and remote regulation of circulation is seen in peripheral tissues. Local regulation can be seen during the process of angiogenesis, while global regulation is a function of the sympathetic nervous system. Neurotransmitters such as NE, adenosine triphosphate (ATP), and neuropeptide Y (NPY) function as vasoconstrictors, while acetylcholine (ACh) and calcitonin gene-regulated peptide (CGRP) are released by parasympathetic nerves to mediate vasodilation [41]. Contrastingly, endothelium-derived relaxing factor nitric oxide (NO) and constriction factor endothelin allow for the functionality of vessels in physiological conditions [41]. External stimuli such as stress,
trauma or pain activates the sympathetic nervous system, which can send signals to vessels within adipose to increase vascular resistance. This effect is mediated by alpha-adrenoreceptors.

NE release can result in the activation of $\alpha_1$-adrenergic receptors within smooth muscle cells in order to constrict vessels. It may also act on $\alpha_2$-adrenergic receptors within the endothelium, resulting in vasodilation through the release of NO as an endothelium-derived relaxing factor [41]. These two receptors can regulate each other through a negative feedback loop to maintain homeostasis within the vessel.

NO is a peripheral neurotransmitter and neuromodulator. Additionally, it can penetrate smooth muscle cells to induce relaxation. NO is synthesized by endothelial NO synthase (eNOS) whereby L-arginine is converted to L-citrulline [42]. NO has properties that prevent its storage in lipid-lined vesicles, so it is synthesized on demand and is diffused from nerve terminals rather than being stored in synaptic vesicles [43]. This may also suggest that NO exhibits neuromodulating effects, as it can influence both neuronal and non-neuronal cells following its release.

**Metabolic Changes as a Result of Aging**

Many metabolic pathologies are associated with aging, as visceral fat mass generally increases with age in response to reduced physical activity and a lower basal metabolic rate, while scWAT mass tends to decrease due to ectopic lipid deposition [11, 44]. The diminishing depot size correlates with an increase in insulin resistance, which puts older individuals at further risk by promoting the development of diabetes [45].

This shift in lipid storage from the scWAT to the visceral abdominal depot is thought to occur due to a decline in progenitor cell function and the accumulation of
senescent adipose tissue cells [46]. Senescent cells arise from the gradual shortening of telomere DNA sequences until cell division is no longer possible [11]. Senescent cells lack the ability to divide in response to metabolic stress, so they limit the proliferation of healthy adipocytes within the depot.

The reduction in scWAT depot mass may also be due to an upsurge of ‘mesenchymal adipocyte-like’ cells [47] within the depot. These cells have a reduced capacity for lipid storage, which results in ectopic lipid deposition [44]. This shift may also be caused by an increase in the presence of circulating reactive oxygen species (ROS) due to mitochondrial dysfunction [44, 48]. This dysfunction occurs because the protein activity within the mitochondrial membrane declines overtime and results in the decline in oxidative phosphorylation during cellular respiration [49]. The electron transport chain then begins to leak electrons, which will reduce the surrounding oxygen molecules and produce ROS such as superoxide anions [49]. These ROS are harmful because they contain an unpaired electron, which will cause them to nonspecifically react with surrounding macromolecules. Excessive ROS accumulation is detrimental and can react with NO to produce peroxynitrite, which can lead to cellular apoptosis, hypertension, protein modification and neural cell damage [50].

It is in the periphery that dysfunction within the metabolic circuitry can be seen at its earliest and most severe. Since aged scWAT cells have reduced sensitivity to insulin, this increases their susceptibility to the development of diabetes. Rates of obesity, the primary precursor of diabetes is steadily rising. According to a recent study, the prevalence of obesity in the U.S. has increased from 30.5% to 42.4% in the last 18 years [51]. Worldwide, obesity rates have tripled since 1975 [52]. Obesity has been associated
with an increased risk for cardiovascular disease mortality and morbidity, particularly with central deposition of adipose tissue [53]. Additionally, obesity increases the risk of hypertension. This is because an increase in the size and number of cells is associated with a higher necessity for blood oxygen and nutrients [54]. Higher blood pressure is associated with a lower total peripheral resistance and decreased perfusion to peripheral tissues [55, 56]. Chronic hypertension can lead to stroke, left ventricular hypertrophy, myocardial infarction, and heart failure [53, 55]. Additionally, the total blood volume and cardiac output are increased in obesity and cardiac workload is heightened [53]. These factors decrease vasodilation and increase vasoconstriction of peripheral vessels in obese individuals [56]. Diabetes is also associated with neural dysfunction, including peripheral neuropathy.

Peripheral neuropathy involves the denervation of peripheral tissues through the gradual dieback of nerves beginning in the skin. Neuropathy results in pain, loss of sensation and discomfort [57]. When neural connections are severed as a result of neuropathy, important endocrine cascades are halted. The neuropathic phenotype can extend deep into peripheral tissues, eventually reaching the adipose tissue. Denervation of WAT, specifically, leads to a loss of proper metabolic control, and an increase in tissue mass and adipocyte cell number [2]. Over 50% of diabetic individuals experience peripheral neuropathy, which results in many limb amputations [58].

To investigate changes in adipose innervation as a result of age, protein expression of the pan-neuronal marker PGP9.5 can be compared in young (12wk) and old (65wk) C57BL/6 mice. These studies show that total innervation decreases significantly (p=0.0031) with age [59].
Vasa nervorum are small blood vessels that penetrate nerve bundles to supply them with nutrients [60, 61]. These vessels can be intrinsic, meaning that they supply blood to the connective tissue surrounding the myelin sheath of each axon within the bundle [60]. If they are extrinsic, this means that they provide blood to the nerve bundles themselves [60]. Blood flow to intrinsic capillaries decreases with age [62], which may suggest that these vessels are associated with the development of neuropathy in peripheral tissues.

Additionally, aging results in a reduction of blood vessel elasticity and compliance due to an increase in collagen content in the vessel walls [63, 64]. This stiffening of the blood vessels reduces their ability to pump oxygen and nutrients to peripheral resistance vessels, which are responsible for regulating blood flow. This causes an increase in circulating systolic blood pressure and may result in cardiovascular disease overtime [63, 65]. The stiffening of vessels also increases one’s susceptibility to atherosclerosis and chronic heart failure [66].

Aging also results in reduced levels of NO bioavailability [67]. This reduces the ability of the vessel to relax and may result in endothelial damage overtime. In healthy individuals, NO also works in concert with VEGF to promote angiogenesis [67]. Thus, age-associated endothelial dysfunction may not only limit adipose tissue blood flow, but also the angiogenic capacity of the tissue [67]. Since mitochondrial dysfunction results in leakage of superoxide ROS molecules during cellular respiration, these molecules readily bind to whatever NO is present within the endothelium of vasculature to create peroxynitrite. This peroxynitrite is toxic to endothelial cells and can result in hypoxia in surrounding tissues [67]. Microenvironment hypoxia where vulnerable cells
(preadipocytes) are located may result in long-term dysregulation of individual cells [48]. This hypoxia may be compounded by inflammation present in aged tissues.

Furthermore, aging can result in the loss of some autonomic nerve projections and a reduction in receptor responsiveness [11]. This may promote an opposite pathway, in which neuropathy precedes vascular dysfunction. Using this thought process, nerve dieback would be associated with a reduction in vascular control and basal tone, which would promote hypoxic conditions within the depot. This would cause a positive feedback loop in which both systems reduce the other’s functionality.

Although there have been many scientific breakthroughs that facilitate the ability of individuals to lead longer lives, this does not necessarily mean that people are healthy in their old age. Aging results from the accumulation of molecular and cellular damage over time, and these changes can dramatically decrease one’s quality of life.

**Pressure Myography Versus Wire Myography**

There are many techniques that can be used to test the mechanisms behind aging-related vascular dysfunction in i-scWAT. There are two options available to support this goal – wire myography and pressure myography.

Wire myography is an in vitro technique that measures vessel wall tension under isometric conditions [68]. After the chosen vessel is isolated from the surrounding tissue, it is transferred into a myography chamber containing aerated physiological salt solution (PSS) at 37 °C [69, 70]. Then, two small wires made of tungsten or stainless steel are inserted in parallel along the interior of the vessel. The ends of these wires are secured by mounts on either side of the bath. One of the mounts is paired with a micro-positioner to set the vessel diameter, and the other is paired to a force transducer [71]. The vessel is
then equilibrated for 30 minutes under these conditions before the normalization phase, during which the myograph is set to a pressure that reflects the \textit{in vivo} pressure of the vascular bed. Following this, the vessel is pre-constricted using norepinephrine or phenylephrine (PE) before the bath is washed with PSS multiple times. The vasoconstrictor is then added once more before a vasorelaxant such as acetylcholine or calcium is added incrementally in order to obtain a dose-response curve [69, 70]. This curve is typically used to determine the percent of dilation of the vessel over time. Wire myography is also used to measure the tension produced when a vessel’s wall is stretched.

Pressure myography is very similar to wire myography, except it uses a different mounting technique and measures myogenic tone in a different way. To start, the vessel is dissected and isolated using the same methods as wire myography. Following this, a pre-pulled glass cannula is inserted into the end of the vessel and attached via a 10-0 nylon suture. The vessel is then transferred into a vessel chamber containing an aerated physiological solution before another cannula is inserted and tied to the other end of the vessel. The vessel is then left to equilibrate before it, too, is exposed to various vasoconstrictors and vasodilators. The difference here is that the pressure typically is kept constant, and the difference in inner lumen diameter is measured through the use of a USB camera above the vessel chamber. A blind sac method can be performed, in which only one of the cannulas receives a pressure input while the distal end is closed off [71]. This method is used for experiments that do not require a change in pressure. Contrastingly, a constant pressure-flow setup involves a perfusion circuit continuously flowing physiological solution through the vessel [71]. This method can be used to
measure changes to the vessel in response to flow or a change in pressure. Either way, the
goal of these experiments is typically to determine diameter changes in response to
stimuli. For blind sac experiments, the stimulus is the injection of chemicals into the bath
solution. When the stimuli being manipulated is pressure, myogenic tone can be
calculated.

Ultimately, the pressure myography technique has been shown to be superior to
wire myography in resistance vessels for a multitude of reasons. The first of benefit is the
improved physiological conditions that is permitted through the use of pressure
myography [71]. Since the two glass cannulas only are inserted at the ends of the vessel,
the majority of the smooth muscle and endothelial cells are left intact. Since wire
myography involves the insertion of two thin wires throughout the length of the vessel,
this results in increased manual manipulation of the vessel, as well as an increased
probability that the wire could denude endothelial cells and even puncture the lumen.
This is immensely important for these types of experiments, as the functionality of the
vessel is meant to reflect the in vivo behavior of the vascular bed [70]. Additionally, the
vessel segment can be pressurized in a way that preserves the cylindrical configuration of
the vessel under isobaric conditions [68]. This is because uniform pressure is applied to
the inner vessel wall as opposed to radially stretching the vessel in one direction, which is
performed during wire myography experiments [71].

Another benefit to this system is that it can be used more effectively on smaller
arteries compared to wire myography. Both pressure and wire myography can be
performed on vessels as small as 60 µm, but wire myography is typically only done on
vessels with an internal diameter between 500 µm and 10 mm [68, 70, 72]. The vessels
intended for this experiment, known as the subcaudal epigastric artery and vein (SCEA/SCEV), are approximately 62 μm in diameter. The vessel diameter can be determined by comparing them to a first order mesenteric artery, which is 62.5 μm [73]. Their size makes the insertion of two small wires incredibly difficult. The smallest wires that are available for wire myography are 25 μm thick, so if two of these are inserted, this would only provide 10 μm of wiggle room. Since these wires are so thin, they also bend very easily, which reduces one’s capability of successfully inserting them through the length of the vessel without puncturing the lumen. It should also be noted that larger elastic arteries such as the aorta may be too thick to allow visualization of the inner vessel during pressure myography [71].

Vasotracker Overview

Due to the numerous benefits of pressure myography, this method is the most suitable for experimentation on small resistance vessels. There are a few vendors that supply pre-built pressure myograph systems, but most are priced at around $40,000. However, a company called Vasotracker, based in Glasgow, Scotland, has an open-source repository for building pressure myographs. This system can be built at a fraction of the cost of the commercially available alternatives. Not only does this company provide a materials list for building the components of the pressure myograph, but it also includes instructions for putting everything together. They have their own software as well, which is compatible with all of the components of the myograph.

Many components of the Vasotracker myograph must be assembled by hand. This includes the vessel chamber, pressure monitor and a temperature controller. Besides the
cost, another benefit to this system is that it is customizable based on the user’s needs since the code is easily accessible.

**Goal and Objectives:**

The ultimate goal of this process is the assembly of a functional pressure myograph that can be used to test the functionality of small resistance blood vessels.

The first objective of this process is the ordering of all components. To build the Vasotrack pressure myograph and monitor in America, parts from the repository must be outsourced. This is because most of the vendors listed on the repository are located in the United Kingdom, and many do not ship their products to other continents. This involves communication with vendors to receive and fulfill purchase orders. After all of these parts are ordered and delivered, the next step is the assembly of the pressure myograph according to manufacturer instructions.

Following this, the next objective in this process is the installation of the software. This includes Vasotrack (measures changes of vessel diameter), µManager (creates a configuration file for the cameras to make them compatible with Vasotrack), Thorcam (tests the functionality of the Thorlabs CS165MU/M USB camera), Pylon (tests the functionality of the Basler Ace 2 USB camera), Arduino IDE (connects the pressure monitor to the Vasotrack software), and NI-DAQmax (configures the code on Vasotrack). All the software/drivers must be compatible with one another in order for the system to work.

The last objective is preliminary experimentation. This involves the optimization of the vessel dissection, isolation, cannulation, and mounting techniques. It also entails
the performance of preliminary experiments to ensure that all components of the myograph work in synchrony. These preliminary experiments may be done to ensure that tubing does not leak, that the cannula arms are stable, and that the software connects to the software and takes quality recordings of the vessel within the chamber. These steps may be performed multiple times to ensure that future experiments run smoothly.
METHODS

Building the Pressure Myograph

The Vasotracker repository includes files and instructions for building the vessel chamber, pressure monitor and a temperature controller [74]. An external water bath provides more consistency in the temperature of the bath solution, and reduces the exposure of electrical components to liquids, so it can be used instead of a temperature controller. This item was purchased from Cole-Parmer (part number: UX-12105-84). A Grothen G728-2 peristaltic pump can be used to circulate the solution in the vessel chamber. This is the only modification to the assembly instructions on the repository.

![Diagram of the Vasotracker pressure myograph system]

**Figure 1: Vasotracker pressure myograph system.** Schematic diagram showing the core components of the myograph. Arteries are mounted into the vessel chamber and imaged using a USB camera attached to the inverted microscope. The intraluminal pressure of the mounted vessel is measured via two pressure transducers connected to a pressure monitor. These transducers measure the pressure input from two height-adjustable solution reservoirs. The chamber temperature is controlled by a water bath, which is set to 35 °C. Image modified from [68].
Vessel chamber:

The first step in the assembly process is the production of the vessel chamber. The chamber base and insert can be purchased from a manufacturing company called Protolabs. These components are made of POM-C, an engineered plastic that is also known as acetal copolymer [75]. The cannula arm fixtures can be 3D printed with resin or acrylic.

The chamber insert attaches to the chamber base via two M3 thumb screws from Robotshop (part number: RS 664-4637). A hole in the middle of the chamber insert allows for the passage of light from the inverted microscope. A 25 mm glass coverslip from Carolina (part number: 633037) can be superglued to the chamber insert to cover the hole. After this, neodymium disc magnets from Walmart (part number: D316X18N48-100PK) are superglued into the holes along the chamber base. These magnets can also be added to the bottom of the two 3D-printed plumbing holders. These have an opposite polarity to the ones on the chamber base so they can be used to support bent needles that circulate solutions in the bath. The next part of this process is the addition of the cannula arms. The M3 screws from Robotshop are used to connect two mounts to the chamber base. A 3-axis transition stage from Thorlabs (part number: DT12XYZ/M) is placed on each mount in order to hold the cannula arms. These cannula arms are made with varying lengths of metal rods from Thorlabs (part numbers: AP4M3M, MS05R/M, MS1.5R/M, MS3R/M, and MSRA90), M2 screws and nuts from Amazon (part number: YMX-1-BI-BH002), and multiple 3D-printed cannula features.
Figure 2: Vessel chamber assembly. Aerial CAD file depicting the Vasotracker vessel chamber, chamber insert, 3-axis transition stages, and cannula arms. Image modified from [68].

**Pressure monitor:**

To start building the pressure monitor, a Wheatstone amplifier shield from Robotshop (part number: RB-onl-38) is placed on top of an Arduino Uno board from Amazon (part number: A000066). After this, two pressure transducers from Digikey (part number: 26PCCFG5G) are added to the amplifier shield through the use of two 12” jumper wires from Robotshop (part number: PRT:10374). Stackable headers from Robotshop (part number: RB-spa-928) are then placed on top of the Wheatstone amplifier shield to allow room for the transducer connections. The A0 pin1 of the stackable header must then be bent outward to allow for rerouting. After this, a 1602 LCD shield from Amazon can be stacked onto the stackable headers and a female 1-pin jumper wire from Sparkfun (part number: PRT-10374) can be used to connect the A0 header pin from the amplifier shield to the Vin (5V) pin on the top of the LCD shield. This step must be performed because the LCD shield uses the A0 pin for button functionality, while the Wheatstone amplifier shield uses A0 and A1 for the bride inputs. By rerouting this connection, the buttons remain functional, and the other connections are maintained.
charger is then attached to the Arduino Uno board to provide power. In order to program the pressure monitor so that it is compatible with the Vasotracker software, a portion of the Wheatstone amplifier shield code has to be transferred into the Arduino code. After this, the pressure monitor will light up and show the pressures of the input and output transducers. When the pressure monitor is functional, a Lego enclosure can be built to provide user protection from electrical currents, and to prevent dust buildup.

![Figure 3: Pressure monitor assembly. A) Interior of the pressure monitor. At the bottom is an Arduino Uno board, which is connected to a blue Wheatstone amplifier shield, as well as a power source. At the top of the amplifier shield are numerous stackable headers to ensure room for the placement of the two pressure transducers. Attached at the top of the stackable headers is an LCD shield, which encompasses the screen and buttons to provide functionality to the pressure monitor. There are two female 1-pin jumper wires that are used to reroute the electrical circuitry. The red wire serves to prevent system from resetting, and the brown wire serves to reroute the bride inputs. B) A Lego enclosure surrounds the exterior of the pressure monitor to ensure user safety and to protect the inner components from damage.](image)

**Mouse Model**

For this study, i-scWAT tissue should be obtained from both young (12-15 weeks) and old (approximately 65 weeks) C57BL/6 mice ordered from The Jackson Laboratory. For the aged cohort, 65 weeks is preferred because an advanced peripheral neuropathic phenotype is expressed in mice at around this time [2]. This mouse line is
considered as genetically identical [76], which allows for a consistency of results. It also provides a means for experimentation on singular pathways.

**Experimental Plan**

**Vessel Isolation/Cannulation Workflow:**

The first step of a pressure myography experiment is the dissection of the chosen vessel. For the purposes of this experiment, the SCEA and SCEV from a C57BL/6 mouse model are used. These vessels are superficially located underneath the skin of the medial portion of murine hind legs. To collect the vessel, a C57BL/6 mice is first anesthetized using carbon dioxide asphyxiation followed by secondary cervical dislocation. The mouse is then placed in a supine position on a blue surgical pad and left for 10 minutes to allow for blood coagulation. The mouse is then sprayed with ethanol before blunt forceps are used to lift the abdominal skin from the muscle layer. Surgical scissors are used to make an incision in the skin along the midline from below the umbilicus to just above the sternum. The skin is then separated from the underlying muscle until the i-scWAT depot can be exposed. Once the SCEA/SCEV are located, a 6 mm section should be dissected out using surgical scissors, starting at the distal end of the vessel. Forceps should be used to hold the vessel in place after the initial incision to prevent a loss of visibility of the vessels before the medial incision. After the vessels have been removed, the i-scWAT depot can be excised and weighed.
Figure 4: Inguinal subcutaneous white adipose depot anatomy. Cartoon of supine mouse with the i-scWAT depot exposed. To the right is an insert of the vasculature, nerves and lymphatics within the depot. The subiliac lymph node (SiLN) is depicted as yellow and serves as an orientation landmark in i-scWAT. The thoracoepigastric vein (TEV) is depicted as blue. Branches of the subiliac transverse nerves (SiTN) are illustrated as green lines. Image modified from [2].

Following dissection, the vessels are placed on an inverted well plate and covered in HEPES solution to keep them moist. This well plate should then be placed on a dissecting microscope to allow for visualization. Fine forceps and iris scissors may be used to isolate the vessel from surrounding adipocytes. After the removal of the connective tissue, the vessels can be separated. A fresh razor blade may be used to remove branches at the end of the vessel. Any branches along the length of the vessel can be tied off using 10-0 sutures.

1.5 inner diameter glass cannulas purchased from Sutter Instrument (part number: FG-GBF150-86-7.5) can be pulled using a P-30 Sutter Instrument micropipette puller. If the P-30 is used, Heat 1 should be set to 800, Heat 2 should be set to 720 and the Pull Value should be set to 100. Once the cannula has been pulled to the desired diameter, it should be fire polished to provide a smoother surface for cannulation. Vessel cannulation
entails the placement of a pulled glass cannula at the end of the chosen vessel. If a 10-0 suture is loosely tied around the base of the cannula, it can be easily slid over the vessel after cannulation and tightened to ensure a strong seal. The same steps for cannulation

**Figure 5: Vessel cannulation workflow.** First, a well plate cover is inverted, and then the SCEA/SCEV are dissected from the i-scWAT depot. After being isolated from surrounding adipocytes, the vessels are covered in HEPES solution to keep them moist. Following this, cannulas are inserted into either side of each vessel to allow mounting within the vessel chamber.

**Blind Sac Workflow:**

Following the vessel dissection, isolation and cannulation steps, the vessel is mounted onto the cannula arm clamps. The adjustment of the cannula arms into a vertical orientation allows for an ease of placement of cannulas into the clamps. When the cannula arms return to their normal position, the cannulated vessel is submerged in a circulating HEPES solution. See Appendix A for the HEPES recipe. This HEPES solution is heated to 35 °C using a water bath, and should be aerated with a 5% carbon dioxide, 5% oxygen and 90% nitrogen gas mixture to maintain a neutral pH. The
peristaltic pump is used to provide a continuous circulation of the solution within the vessel chamber.

Once the vessel is in the HEPES solution, 1.22 mm ID tubing from Cole-Parmer (part number: WZ-06460) is connected to the distal end of one of the cannulas. This is connected to a piston-less syringe placed above the water bath. This syringe serves as a reservoir for HEPES solution, which flows down the tubing, through the glass cannula, and into the mounted vessel. The tubing attached to the second cannula remains closed using a 3-way stopcock from Cole-Parmer (part number: WZ-30600-02) in order to maintain a constant intraluminal pressure of approximately 70 mm Hg. This pressure can be measured through the use of a Living Systems pressure monitor (part number: PM-4), or a calibrated Vasotracker pressure monitor. Following this, the Vasotracker software can be used to provide real-time visualization of the vessel using a USB camera.

The vessel is then normalized in the HEPES solution for around 30 minutes before 10 µl of 10 mM PE is added to the water bath to constrict the vessel to approximately 70-80% of its original diameter. When a stable constriction is obtained, 10 µl of 10 mM ACh may be added every 10 minutes to obtain a dose-response vasodilation curve. Alternatively, 100 µl of ACh can be added at once in preliminary experiments to ensure vasodilation. After the last dose, rinse with HEPES solution. The recipes for the PE and Ach solutions can be found in Appendix A.

Altogether, this experiment has required the sacrifice of 10 mice. All of these mice were used to practice the vessel isolation and cannulation techniques. Two of these mice were also used to attempt the mounting and experimental portion of the experiment.
The breaking of a cannula arm during experimentation prevented preliminary chemical experimentation from occurring after the mounting phase.
RESULTS

Functionality of the Pressure Myograph

The pressure myograph is functional, as it successfully circulates the HEPES solution, keeps it warm, and is capable of holding mounted vessels in place for visualization. The software is also functional, as it successfully connects with the USB camera drivers to obtain real-time images of the mounted vessel. While experimentation is not yet possible due to the breakage of one of the cannula arms after the mounting step, the Vasotracker software includes a “FakeCamera” option, which measures the inner lumen diameter of a vessel during a pre-recorded trial experiment. This function shows that the software is capable of reading the changes in vessel diameter overtime and converting this information into a reliable dataset.

While the software and components all work harmoniously, there are many things that can be improved upon in future experiments. During this process, a physiological air solution is meant to maintain the pH of the circulating solution. However, since the regulator and air tank are not yet connected, this did not occur. Additionally, the Vasotracker pressure monitor is not yet calibrated, so a Living Systems PM-4 pressure monitor will be required until the calibration guide is available. The tubing connecting the bent needles to the peristaltic pump also tends to leak, so plumber’s tape will need to be reapplied during future experiments to prevent damage to the electrical components of the myograph.

Additionally, the mounting procedure can be improved upon. The only technique that has shown to be successful involves the manual placement of both cannulas into the
cannula arm clamps before lowering them into the solution. Since these cannulas should be tied to the vessel, it can be incredibly difficult to transfer them without tearing the vessel or sliding one of the cannulas out. Many protocols suggest mounting one end of the vessel inside the vessel chamber and then placing the other cannula into the other arm clamp before moving them together and tying off the other end. However, the vessel chamber contains two large transition stages that hold the cannula arms and ultimately obstruct access to the vessel on either side. If the chamber is shifted to a vertical position to provide access on the sides, it will no longer be centered on the inverted microscope. This prevents the light from the microscope to shine through the hole at the bottom of the chamber. If experiments are to commence, this procedure must be optimized in order to prevent damage to the endothelium within the vessel.

Lastly, the isolation and cannulation of the vessel do not occur in truly physiological conditions. If the vessel is placed into the vessel chamber with the circulating solution during these steps, it will move around, be difficult to keep track of, and can be transferred into the HEPES solution due to the circulation caused by the peristaltic pump. Instead, these steps are performed on an inverted well plate cover with room temperature HEPES solution added to keep the vessel moist. This allows access to the vessel at all angles and provides a means for light to pass through for visualization. If this step is to be optimized, perhaps it would be useful to set this well plate over a hot plate set to 35 °C. Still, this design may not be at physiological conditions, as no physiological air solution would be pumped into the HEPES. This is a step that has yet to be optimized, but many journals do not emphasize its importance in their studies at all. In fact, many pressure myography experiments involve the dissection of vessels at 4 °C, or
the placement of the vessel on ice after the isolation. After speaking with an expert in the field, it was recommended that the vessels not be placed on ice so that their physiological properties could be maintained.

It should also be noted that although attempts are made to standardize conditions in pressure myography, it is impossible to mimic in vivo parameters. By removing the vessel from the tethering of the connective tissue, the pressure-length and pressure-diameter relationships are unavoidably altered [70]. The isolation of the vessel also reduces sympathetic and sensory nerve input, as well as typical endocrine communication from adipose. In this way, the results obtained will always be slightly inaccurate.

**Figure 6: Assembly results.** A) The finalized vessel chamber, used to support the mounted cannulas and vessel during experimentation. B) The screen of the pressure monitor once it was connected to the Arduino software and a power source.
**Figure 7: Vessel isolation/cannulation progress pictures.** A) Dissected SCEA and SCEV surrounded by adipocytes. Image taken at 40x. B) SCEA or SCEV isolated from surrounding adipocytes. Image taken at 40x. C) SCEA or SCEV isolated, cannulated and tied with a 10-0 suture. Image taken at 40x. D) Depicts a vessel that has been isolated, cannulated, and mounted onto the vessel chamber. Image taken using the Basler USB camera connected to the Vasotracker software.

**Troubleshooting**

This project may require troubleshooting during development. See Appendix B for a complete summary of the ordering, technological, and experimental hurdles encountered during this process.
DISCUSSION

Although no experimental studies have been performed, there are many possible reasons why aging has been shown to decrease nerve density and neurovascular interactions in aged i-scWAT. Aging-related mitochondrial dysfunction within adipose tissue may lead to an excess production of superoxide anions, which are a type of ROS. Superoxide anions readily bind with NO released from peripheral vessels during sympathetic activation to produce peroxynitrite. Since aged vessels already exhibit a low bioavailability of NO [67], and are less elastic as a result [63, 64], this reaction further reduces the vasorelaxation properties of resistance vessels. Furthermore, this reaction produces a toxic microenvironment within adipose tissue due to the presence of the peroxynitrite, which is detrimental to vascular function in and of itself since it oxidizes cellular proteins, lipids, low-density lipoprotein particles, and causes direct endothelial toxicity [77]. A hypoxic environment forms, which then can lead to neuropathy and further vascular dysfunction. Additionally, since peroxynitrite also damages mitochondrial DNA/RNA [78], this is a self-fulfilling prophecy that promotes continual dysfunction and neural die back.

Of course, the validity of this hypothesis will need to be tested using different mechanistic knockouts and chemicals during pressure myography experiments. Through the use of this handmade myograph, the vascular tone of aged peripheral arteries within the i-scWAT can be quantified and compared with younger cohorts. This data may help determine the cardiovascular effects of neuropathy. Testing the responsiveness of the mesenteric artery within the visceral WAT may also be vital to examine the role of aging
in deeper tissues. Since the visceral fat depot increases in size with age, this may decrease rates of adipogenesis in the depot. This is a novel field of research, and there truly are dozens of paths this research could take. Now that the pressure myograph system is finally built, it can be used for these experiments. Additionally, since the Vasotracker code is easily accessible, it may be altered to fit the needs of future experiments.
FURTHER RESEARCH

The next step in this process will involve preliminary experiments exposing mounted vessels to PE and ACh and measuring changes to the inner lumen diameter. After this, more myography experiments can be performed that go more in depth into influences of aging on blood vessels. This may include comparative aging studies, mechanistic studies, or neuropathic studies in different depots. More experiments/imaging can also be done to determine the rate of reduction of neurovascular interactions in mice overtime. This may help to determine the age at which the prevention of neuropathy is most vital. It may also help to determine which protective factors may reduce the prevalence of neuropathy in aged mice. Since this is the first time this technique has been used in the i-scWAT depot, there are many novel paths that this research can take.

Other studies that may be done as a result of these initial findings are high-fat diet or induced-diabetes studies to determine the role that these pathologies play in neurovascular interactions within peripheral tissues. These can be compounded with an aging component, as obesity and diabetes have been shown to exacerbate the deleterious effects of aging [11]. Additionally, the neural vasculature is also affected by aging [79]. By using pressure myography alongside neuroplasticity experiments, new conclusions can also be made regarding illnesses such as Alzheimer’s. Performing experiments in the brain may also provide further mechanisms by which peripheral neurovascular interactions change overtime.
Other areas of research that could be interesting to focus on are the differences between the mesenteric depot and i-scWAT, as well as the role of PVAT under numerous conditions. Furthermore, by determining which nerves and vessels interact in the murine model, a web can be formed connecting the physiological effects of these interactions throughout the body. Eventually, these findings may even expand into human studies in order to determine a treatment plan for individuals with diabetes, or to prevent neuropathy in the aging population.
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APPENDICES
APPENDIX A

Pressure Myography Protocol (Blind Sac)
June 2021

Instruments/tools:
- Inverted Microscope
- CCD Camera
- Scalpel
- Blunt forceps
- 10 ml syringe
- Vastotracker setup
- Suture thread

Reagents:
- HEPES solution (1 liter)
  - 7.35 g of sodium chloride, 0.402 g potassium chloride, 1.8 g glucose, 0.353 g sodium bicarbonate, 0.054 g potassium phosphate, 0.041 g sodium phosphate, 0.091 g magnesium phosphate, 2.38 g HEPES, 0.264 g calcium chloride (dihydrate), 0.22 g sodium pyruvate, 0.1015 g magnesium chloride hexahydrate
    - Prepare fresh on day of experiment.
    - Place bottle into water bath, set to 35 °C.
    - Save 100 ml for the washing step at the end of the experiment.
- Acetylcholine/ACh solution (10 mM)
  - Add 0.018 g of acetylcholine into 10 ml of distilled water.
  - Add to vessel chamber when ready to use.
    - Can aliquot 0.5 ml of stock solution and freeze it for later use.
- Phenylephrine/PE (10 mM)
  - Add 0.020 g of phenylephrine into 10 ml of distilled water.
  - Add to vessel chamber when ready to use.
    - Can also increase by 1 µM in 10-minute intervals until vessel contracts by 80%
    - Can aliquot 0.5 ml of stock solution and freeze it for later use.

Preparation of Perfusion Chamber and Sutures:
1. Rinse chamber with Milli-Q followed by dissection solution for 5 min each. Load the chamber with 2 ml of HEPES solution. Keep solution circulating within chamber through the use of a peristaltic pump.
2. Prepare two sutures with a half-knot each using blunt forceps under a dissecting microscope and set to the side. If the area has some airflow, place sutures on double sided/rolled tape to keep them in place.
   a. Ensure that they are not so tight that you cannot slide them onto the cannula, but not so loose that they will unravel when you pick them up.
   b. Make a few, as you may not be able to get them off the vessel if something happens.
**Tissue Isolation Procedure:**
4. Use dissection scissors to dissect superficial caudal epigastric artery (SCEA) from inguinal scWAT depot. Dissect depot of chosen side and weigh.
   a. Refer to tissue isolation protocol for detailed dissection instructions.
   b. Try to stick to same side for every experiment.
5. Isolate and dissect an approximate 6 mm section of SCEA (remove surrounding adipocytes under a dissection microscope).
   a. Refer to tissue isolation protocol for detailed isolation instructions.

**Pipette Pulling Procedure:**
6. Obtain a 1.5x.86mm capillary tube.
7. Turn on pipette puller.
   a. Set Heat 1 value to: 800
   b. Set Heat 2 value to: 720
   c. Set pull value to: 100
8. Loosen the upper and lower clamping knobs.
9. Place the capillary tube in the V-groove within the upper clamp. Place it so that only 1 cm of the tube is visible above the clamp. Tighten the clamping knob.
   a. If you want two shorter pipettes, you can adjust the position of the capillary tube so that the portion that is visible is the same length as the portion within the slide bar.
10. Pull the lower clamp upwards until it no longer moves before tightening the clamping knob.
11. Press the pull button (heating filament will turn red before the lower clamp falls).
12. Wait for the pipette to cool off before releasing the clamps and obtaining pulled pipettes.
   a. Two cannulas are needed for this experiment.

**Myography Procedure:**
13. Cannulate one end of the vessel onto one of the pulled glass pipettes using dissection forceps and secure a 10-0 suture around the vessel. Ensure that the vessel stays wet the whole time. Mount onto vessel chamber and submerge in around 2 ml of HEPES solution.
15. Bring the other cannula closer to vessel and attach using second suture under inverted or dissecting microscope.
16. View vessel using 10x objective. Can use either dissecting or inverted microscope.
17. Connect the inlet (right) valve with P1 tubing from the pressure monitor. Close stopcock. Connect inlet valve with small piece of tubing and attach it to the right cannula. P1 should be connected to tubing attached to a solution reservoir (a syringe without a piston).
18. Connect the left valve of the chamber with the P2 tube on the pressure monitor. Close the stopcock unless doing myogenic tone experiments. Open the input valve and adjust the height of the syringe until the pressure is 70 mmHg (as read by pressure transducer).
19. Start perfusion of chamber bath with 100 ml of HEPES, warmed with a water bath (35°C, equilibrated with gas mixture: 5% CO2, 5% O2 and 90% N to maintain neutral pH and adequate oxygenation). Let the vessel equilibrate to this temperature for 30 minutes.
   a. Label “Ref” in Vasotrack when vessel is equilibrated.
   b. Adjust “rec intv” value for time desired between pictures.
   c. Adjust “smooth” value for lumen diameter.
   d. Turn filter on, to prevent recognition of outliers/extraneous items (bubbles, sutures, miscellaneous objects within the frame)
   e. If the vessel is not completely isolated, set ROI to match visible inner lumen.
   f. This solution should circulate within vessel chamber continuously.

22. Use Vasotrack software to monitor and record inner vessel diameter.

23. Add 10 μl PE solution to HEPES to pre-constrict the vessel. When a stable constriction has been obtained, perform a cumulative concentration-vasodilation response curve (CRC) by sequential addition of 10 μl of acetylcholine After last dose, rinse with HEPES solution.
   b. Use label function on Vasotrack to record inputs of PE and ACh.
   c. Aim for constriction of around 80% when adding PE.
   d. To obtain preliminary data, can also add 100 μl ACh all at once to visualize adequate contraction without stepwise addition.
**APPENDIX B**

**Troubleshooting**

One of the most challenging items to order was the vessel chamber base and insert. Both of these parts had to be CNC machined out of POM-C. These parts can be manufactured by a company called Protolabs. This is the company that was recommended on by Vasotracker. For some reason, the CAD files on the repository were not compatible with Protolab’s software, so it was decided that new CAD files would need to be developed. Since this would take weeks, as well as an influx of money, it was decided to look elsewhere for these parts. The IMRC at the University of Maine offered to make the vessel chamber and insert at a fraction of the cost of Protolabs. Unfortunately, due to COVID-19 and tense relations with China at the time, POM-C was backordered for weeks. No alternative materials would provide what was required for the functionality of these parts, and other vendors proved to be much more expensive, so Protolabs seemed to be the only option. Fortunately, a representative at Vasotracker was willing to transfer a cart with all of the specifications for CNC machining, without the use of CAD files. This included all of the threading depths and instructions to manufacture the components. After a purchase order was created, it was soon discovered that Protolabs only took credit card payments through their website. Once the payment finally went through, another hurdle arose. Since the account was associated with the University of Maine, a tax exemption form for the state of Maine was required to remove the tax charges. The payment was made by the Ohio State University (OSU), so their ordering team only had access to the tax exemption forms for Ohio. This meant that the previous order had to be cancelled and placed under a new OSU affiliated account. Following this, the order finally went through. It was only after these parts were received that the main component of the pressure myograph could be assembled.

During the building process, another hurdle was encountered while assembling the pressure monitor. After the apparatus had been built, it was plugged into a power source and turned on. The second time the power source was connected, the screen no longer lit up, and some of the lights within the Arduino Uno board failed to glow. New replacement parts were ordered, and it was rebuilt several weeks later. After looking at the original pressure monitor, it was discovered that the outputs of the pressure transducers were not in the same order as the inputs to the amplifier shield. This caused the system to short-circuit. Since the original pressure monitor was not encased by an enclosure, dust could also have settled within the circuitry and reduced its functionality.

The budget for this project was further implicated after neither of the spare computers on hand were compatible with the specifications for the Vasotracker software. This software required 8 GB of RAM and a Microsoft Windows 10 Pro 64-bit operating system. The first computer did not support 64-bit versions of Windows, and only had 4 GB of RAM. The second spare computer was an Apple device, which was inherently incompatible with the Vasotracker software. After multiple failed attempts, a new computer was purchased through the IT department at UMaine. However, there were no ethernet hookups available in the lab to set up the internet. The IT department helped to set it up using an external Wi-Fi adapter in a different location. After the computer was connected
to the internet, the software was installed. This came with its own issues, as the installation manual for Vasotracker did not include clear instructions about the retrieval paths.

After the software was installed, the USB cameras were connected. The Thorlabs camera that was suggested on the repository had recently been taken off the Thorlabs website and replaced with a newer version. This was purchased with hopes that it would still connect to the software. It didn’t. The Vasotracker representative attempted to connect this new camera but was ultimately unsuccessful. He suggested the purchase of a new camera from Basler. He was positive this one would work since they had been using it in their lab. This camera did not work either. The original Thorlabs camera was not compatible with µManager or Vasotracker but was compatible on Thorcam. Contrastingly, the Basler camera connected to Pylon and µManager, but would cause the Vasotracker software to crash when it was selected as the source. It took many months and meetings with the representative at Vasotracker to figure out what was wrong. It wasn’t until 6 months later that it was discovered that the code for the Basler camera included the wrong serial number.

Once the Basler camera began to work, it was discovered that it did not fit in the hole of the inverted or dissecting microscopes. This would require the purchase of a mount, but since time was limited at this stage, it was held in place using duct tape and foam. Since this was relatively unstable, it was difficult to visualize the vessel. This was exacerbated by the fact that the inverted microscope had a slide holder that kept the contents of the vessel chamber just out of focus. After this, it was decided to move the camera to the dissecting microscope instead. This was mounted in a similar way, but it had even fewer angles of visualization. There was only one angle that worked, and it showed the vessel on the screen as horizontal instead of vertical, which was required by the software to recognize the lumen diameter. To proceed with experiments in the future, a camera mount must be installed to obtain quality images and to visualize the vessels at the right angle. Additionally, the slide holder on the inverted microscope must be removed if it is to be used during experiments.

Another hurdle that was experienced during the data collection process was the air conditioning in the room. This was constant, and very strong. During the experimental process, the wind would blow away sutures that had been pre-tied, as well as affect the tying process itself. This was a constant occurrence, as the width of the 10-0 sutures is similar to that of a human hair. After multiple failed attempts, it was discovered that the sutures needed to be stuck to tape to prevent them from flying away. The tying of the vessels with these sutures also proved to be challenging, as one pair of the fine forceps used during vessel cannulation was bent at the end. This reduced the grip of the forceps and made it extremely challenging to tie them at close range. After a while, it was decided to make ties with extra long tails in order to tighten them manually. This proved to be very helpful but required the waste of many centimeters of good suture thread.
AUTHOR’S BIOGRAPHY

Bailey Michele Woodard was born in Bangor, Maine on June 30th, 1999. She was raised in Parkman, Maine and graduated from Piscataquis Community Secondary School in 2017. After graduating, Bailey decided to attend the University of Maine to pursue a bachelor’s degree in biology with a pre-medical concentration, as well as minors in psychology and neuroscience. She was also a member of the Honors College. During her time at UMaine, Bailey was a psychology tutor, lead resident assistant, and student lab manager in the Townsend Neurobiology lab. Bailey graduated in the spring of 2021 with a B.S. in Biology.

Upon graduation, Bailey plans to take a gap year before applying to dental school.