Cardiovascular diseases (CVDs) are the leading cause of pre-mature mortality and disease burden globally (Roth et. al, 2020; Mc Namara et. al, 2019). Hypertension, high blood pressure, is a significant risk factor for CVD, and is the most prevalent risk factor. One of the strongest predictors of hypertension is the high dietary sodium intake (Tran et. al, 2021). The World Health Organization (WHO) recommends that individuals ingest less than 5 grams of dietary salt (2 grams of sodium) per day to lower the risk of developing cardiovascular disease and hypertension3,6. However, the diet of many individuals in western society exceeds this recommendation, increasing their risk of developing cardiovascular diseases4. The major sources of salt are processed foods, ready-made meals, and salt added during food preparation, cooking, and at the table (Wang et. al, 2020). This increase in dietary salt leads to an increase in blood pressure, which can lead to the development of cardiovascular diseases (Wang et. al, 2020; Grillo et. al, 2019; O’Donnell et. al, 2015).

Babcock et. al (2019) discuss the past belief that only the kidneys regulated long-term blood pressure through the regulation of renal sodium excretion. This belief led to researchers such as Mente et. al (2018) to claim that sodium has a pathological role when intake is low or high and a physiological role when sodium intake is moderate. These studies only looked at sodium excretion in urine and they also did not study populations that were considered healthy. Instead, majority of individuals that were tested had underlying health conditions and their kidneys did not function properly. However, more recently, Olde Engebrink et. al (2015) and Casale & Crane (2022) identified that sodium is regulated in a 2-compartment model with sodium storage that can occur in the skin interstitium, where glycosaminoglycans (GAGs) bind and inactivate sodium and thus remove it from renal sodium excretion to help the body regulate sodium levels and therefore, blood pressure. High sodium diets increase the amount of sodium content in the skin, leading to an increased expression in both skin glycosaminoglycan content and XYLT-1 (xylosyltransferase 1), an enzyme that that initiates GAG synthesis. This increase is thought to be the main driving force behind skin sodium accumulation during a high sodium diet (Wenstedt et al, 2018).

Glycosaminoglycans (GAGs) are molecules found throughout the body, including skin, joints, blood plasma, and the mucous membrane of various organs (Casale & Crane, 2022). GAGs are large, negatively charged linear polymers consisting of disaccharide unit repeats and are responsible for storing sodium (Casale & Crane, 2022). Specific combinations of these repeating units result in different types of GAGs, such as heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronan, with heparan sulfate GAGs being the most prominent on endothelial cells followed by chondroitin sulfate and hyaluronan GAGs (Olde Engberink et. al, 2015).

The endothelium surface layer (ESL) is a dynamic layer on the luminal side of the endothelial cells that is in continuous exchange with flowing blood. It comprises a network of glycoproteins, adsorbed plasma proteins, and proteoglycans to which glycosaminoglycans (GAG) chains are attached forming the glycocalyx (Olde Engberink et. al, 2015; Wenstedt et al, 2018). The ESL is in direct contact with plasma sodium and therefore, could function as the first sodium buffer before sodium enters the interstitium (Wenstedt et al, 2018). The negative charges of the ESL automatically attract ions of the opposite charge when they are located within an electrolyte solution, such as blood. Because sodium is the most abundant cation in circulating blood, sodium forms a so-called ion atmosphere around the endothelial cell and ESL. Considering the sodium-binding properties of GAGs that makeup the glycocalyx, it is conceivable that the attracted sodium ions are bound and osmotically inactivated by the glycocalyx in the ESL (Olde Engberink et. al, 2015).

An impaired ESL may also facilitate leakage of glycosaminoglycans into the interstitium. Increased leakage of glycosaminoglycans to the skin together with increased skin glycosaminoglycan synthesis may, therefore, serve as the explanation for a sodium-induced increase in tissue glycosaminoglycan content and concomitant sodium accumulation when ESL perturbations are present (Wenstedt et al, 2018).

The ESL also plays an important role in mediating shear-induced nitric oxide (NO) production through mechanotransduction and activation of the enzyme, endothelial nitric oxide synthase (eNOS). Due to its location, the ESL must be considered a possible sensor of fluid mechanical shear stress that can distribute force to other regions of the endothelial cell where transduction to biomolecular signals may occur (Pahakis et. al, 2007). Apart from regulating mechanotransduction, the ESL can modulate NO availability by increasing sodium transport into the endothelial cell (Olde Engberink et. al, 2015). A study conducted by Pahakis et. al (2007) discovered the epithelial sodium channel (EnNaC) on the endothelial luminal surface. It was shown that this EnNaC regulates endothelial nanomechanics and subsequently affects NO production. By enhancing sodium influx, the EnNaC increases mechanical stiffness of the endothelial cellular cortex (Jeggle et. al, 2013; Pahakis et. al, 2007). The stiffness of this 50- to 100-nm layer, which mainly consists of actin "laments, subsequently modulates endothelial NO synthase activity and NO production, where an increasing stiffness attenuates NO production (Jeggle et. al, 2013; Kusche-Virog et. al, 2010). The density of EnNaCs on the endothelial surface is regulated by aldosterone and plasma sodium concentration. A rise in plasma sodium concentration increases EnNaC density, which in turn, increases sodium uptake, stiffens the endothelial cellular cortex, and subsequently, leads to diminished NO production (Kusche-Virog et. al, 2010; Korte et. al, 2012). An increase in sodium delivery to the endothelial cell as a result of an increase in sodium intake could, therefore, lead to an increase in vascular tone (Olde Engeberink et. al, 2015).

Shear-induced nitric oxide production is a hallmark of endothelial mechanotransduction that is a significant marker of vascular tone as it is the most powerful physiological regulator of endothelial Nitric Oxide Synthase, leading to rapid rises in NO (Sprague et. al, 2010; Pahakis et. al, 2007). Inactive eNOS is bound to the protein caveolin. When intracellular levels of Ca2+ increase, eNOS detaches from caveolin and is activated and goes on to produce NO (Sandoo et. al, 2010). Nitric oxide is a labile, lipid soluble gas that utilizes the enzyme eNOS, which catalyzes the amino acid L-arginine to L-citrulline, with NO as a free radical by-product. These are what make NO (Green et. al, 2004; Sprague et. al, 2014). It is important to consider that the production of NO from the conversion of L-arginine through the eNOS enzyme can often become compromised, such as in cardiovascular diseases, and the reduced availability of NO in the body limits the ability of the arteries to properly dilate. The body, however, counteracts this effect through the activation of other redundant dilation pathways, such as prostacyclin (PGI2), which plays a compensatory role in dilation of the vessel when NO is reduced or blocked (Sandoo et. al, 2010).

The maintenance of a healthy endothelial phenotype relies on a delicate balance between NO production and reactive oxygen species (ROS) formation (H2O2 and O2-), both of which are crucial to the maintenance of cellular redox potential and redox-related cell signalling (Lee et. al, 2017). Shear stress exerted by laminar blood flow in the ESL increases NO availability, while reducing ROS production. Shear stress protects endothelial redox homeostasis and counteracts endothelial dysfunction. ROS may uncouple the eNOS-catalyzed reduction of molecular oxygen from the oxidation of L-arginine, resulting in the production of the ROS superoxide anion (O2-)instead of the reducing NO. Alternatively, ROS may react with NO directly, reducing its bioavailability (Zhao et. al, 2015; Schulz et. al, 2011; Förstermann & Münzel, 2006). When increased oxidative stress and endothelial dysfunction are encountered, the expression of eNOS increases to compensate. The demonstration of endothelial dysfunction in the presence of increased expression of eNOS indicates that the capacity of the enzyme to produce NO may be limited, and the concept that eNOS itself can be a superoxide source and thereby contribute to endothelial dysfunction (Schulz et. al, 2011; Joyner & Green, 2009). Decreased NO availability, secondary to enhanced NO degradation by ROS can tip the redox balance and cause impaired NO-mediated signalling, an early hallmark of endothelial dysfunction (Lee et. al, 2017; VanBavel, 2007). The buildup of these ROS can then lead to a decrease in bioavailability of NO, leading to endothelial dysfunction.

There is also evidence that differing sodium levels alter endothelial function and NO production1,4. During high sodium intake, the glycocalyx becomes deteriorated or “stiff” due to a lack in heparan sulphate residues and thus exhibiting a reduced sodium buffer capacity. This then interrupts cell signals, such as the mechanical stimulus which triggers eNOS to produce NO (Oberleithner, 2011). Oxidative stress due to an increase in ROS generation decreases NO bioavailability and sodium induced endothelial dysfunction (Edwards & Farquhar, 2015). This decrease in NO would then increase arterial blood pressure because when there is a decrease in NO bioavailability, the blood vessels become less responsive to the normal signals that regulate blood flow, leading to an increase in vascular resistance and a rise in blood pressure (Hermann et. al, 2007; Edwards & Farquhar, 2015).

Exercise improves endothelial function through increased in shear stress mediated NO production (Goto et. al, 2007). The repeated induction of eNOS activity that occurs during exercise training, might prolong the half-life of NO by reducing its degradation by free radicals or by directly decreasing free radical production (Green et. al, 2004). However, exercise also leads to immune activation through increased movement of blood and lymph and catecholamine induced increases in immune cell function (which contains white blood cells). The increased shear and catecholamine responses increase cell motility and more immune cells from lymph nodes and the spleen migrate into the bloodstream (Cerqueira et. al, 2020). This increased immune response then leads to elevations of ROS present. The glycocalyx can also be modified after acute perturbations like an increase in blood flow and following this perturbation, it may be contributing to changes in how well blood vessels dilate. A study conducted by Sapp et. al (2019), predicts that a high intensity exercise bout induces acute glycocalyx shedding due to increased amounts of ROS due to an increase in oxidative stress. The endothelial function decreases as a result as the glycocalyx plays a major role to shear-mediated NO production. As the glycocalyx sheds, NO bioavailability decreases along with endothelial function (Sapp et. al, 2019; Kröpfl et. al, 2021). In our study, the level of exercise will only be moderate, so we may not see immune response induced glycocalyx shedding. Locally mediated blood flow can also affect the thickness of the glycocalyx. When blood flow is increased, the shear stress from the flowing blood can cause the glycocalyx to become less thick or even to be shed from the endothelial surface, allowing for decreased dilation of the blood vessel (Kröpfl et. al, 2021).

Flow-mediated dilation (FMD) is used as a non-invasive approach to observe and analyze endothelial function and endothelium-derived NO bioavailability, particularly that of the brachial artery (Thijssen et. al, 2011; Green et. al, 2011). The FMD technique increases blood flow through an artery to cause smooth muscle relaxation and subsequent dilatation on the principal that the increased blood flow produces shear forces on the endothelium and is transduced using mechanoreceptors and subsequently stimulates endothelial cells to release NO (Sandoo et. al, 2010; Harris et. al, 2010). Reduced dilatation following an increase in shear forces is representative of impaired NO bioavailability and therefore, FMD is a good surrogate marker of NO bioavailability (Green et. al, 2011; 2004). This method involves direct imaging, using a Doppler ultrasound, of large artery dilator responses to shear-stress-induced FMD consequent to a brief period of limb ischaemia, a decrease in blood supply to the tissues in the limb. Assuming the occluding cuff is placed distal to the scanned artery and that the period of ischaemia does not exceed 5 min, the increase in arterial diameter in response to this stimulus is almost exclusively mediated by NO (Green et. al, 2004; Thijssen et. al, 2013).

When pairing FMD with exercise, multiple studies have discovered that there is a biphasic change in FMD after exercise. This is where there is a decrease in endothelial function and therefore, a decrease in the FMD response when performed immediately after a 30-minute bout of exercise (Dawson et. al, 2013). A study conducted by Johnson et al. 2012, found that FMD decreased immediately following 30 minutes of either moderate or high intensity cycle training. The FMD response then returned to baseline levels by the 60-minute mark post exercise. Nitric oxide bioavailability is decreased and there is a development of oxidative stress during exercise which likely contributes to an immediate decrease in FMD after a single exercise bout (Dawson et. al, 2013). When sodium levels are increased, it has been demonstrated to impair endothelial function, as assessed via FMD. When paired with exercise, we predict that the exercise will exacerbate the sodium induced endothelial dysfunction and prevent the artery to recover and return to baseline endothelial function when the final FMD is conducted.

Upon completion of this study, it is hoped that when there is an increase in sodium levels, the endothelial function will be affected especially in vascular systems where the glycocalyx has been modified by increases in blood flow. It is also hoped that arterial blood pressure will be increased systemically. We will alter the glycocalyx in an isolated limb to test whether this salt sensitivity happens in young adults. If these results are validated, future studies can assess differing sodium levels in the ESL to help determine when the endothelial function and arterial blood pressure become affected. With this information, individuals will then be able to adjust their dietary sodium intake to a level which reduces the risk of cardiovascular disease and hypertension.