Short-Term Ingestion of Virgin Coconut Oil Improves Endothelial-Dependent Dilation but not Exercise-Mediated Hyperemia in Healthy Young Adults

by

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Abstract

Nitric oxide (NO), a powerful vasodilator produced in the endothelium, can be rapidly converted into toxic peroxynitrite by reactive oxygen species (ROS). Virgin coconut oil (VCO) is high in antioxidants (polyphenols) that reduce the action of ROS, and may subsequently increase flow-mediated dilation (FMD) and exercise-mediated hyperemia. This has never been assessed in humans. We investigated whether 30 $ml \cdot day^{-1}$ VCO for 4 weeks would improve popliteal artery (PA) FMD, improve the PA hyperemic response to moderate-intensity cycling, and alter plasma total antioxidant content (TAC) in healthy adults (10♂, 22±2 years). Short-term VCO ingestion increased FMD (Pre-VCO, 4.9±0.9%; Post-VCO, 5.7±1.2%; $p < 0.001$), but not five-minute mean post-exercise hyperemia (Pre-VCO, 102±75 $ml \cdot min^{-1}$; Post-VCO, 124±83 $ml \cdot min^{-1}$; $p = 0.28$), or plasma TAC (Pre-VCO, 96.0±15.1 $ng \cdot ml^{-1}$; Post-VCO, 96.8±17.0 $ng \cdot ml^{-1}$; $p = 0.88$). These results indicate that VCO may increase NO bioavailability, and that NO contributes relatively little to the exercise-mediated hyperemic response.

Word Count: 149

Keywords: flow-mediated dilation, nitric oxide bioavailability, exercise-mediated hyperemia, popliteal artery, total antioxidant capacity

List of Abbreviations and Symbols Used

ADMA	asymmetric dimethylarginine
B-mode	brightness mode
BMI	body mass index
Ca^{2+}	calcium
Ca^{2+}-ATPase pump	calcium-adenosine triphosphatase pump
cGMP	cyclic-guanosine monophosphate
CO	copra oil
Cu/Zn-SOD	copper/zinc superoxide dismutase
CV	cardiovascular
CVD	cardiovascular disease
DDAH	dimethylarginine dimethylaminohydrolase
ELISA	enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
FMD	flow-mediated dilation
%FMD	percent change (from baseline) of peak FMD
5'GMP	5-guanosine monophosphate
GNO	ground nut oil
HDL-C	high-density lipoprotein cholesterol
5HPO	five-times heated palm oil
HR	heart rate
HRR	heart rate reserve
ICAM	intracellular adhesion molecule
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
L-NMMA	L-N^G-monomethyl arginine
MCFA	medium-chain fatty acid
Mn-SOD	manganese superoxide dismutase

NADPH	nicotinamide adenine dinucleotide phosphate
NE	norepinephrine
NO	nitric oxide
NTG	nitroglycerin
O_2^-	superoxide radical
PA	popliteal artery
PAR-Q	physical activity readiness-questionnaire
PDE-5	phosphodiesterase-type 5
PGI_2	prostacyclin
Q	cardiac output
RBD	refining-bleaching-deodorizing
ROI	region of interest
ROS	reactive oxygen species
SOD	superoxide dismutase
SR_{AUC}	shear rate area under the curve
SV	stroke volume
TAC	total antioxidant activity
VCAM	vascular cell adhesion molecule
VCO	virgin coconut oil
VLDL-C	very low-density lipoprotein-cholesterol
VSM	vascular smooth muscle

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Chapter 1: Introduction

Cardiovascular disease (CVD) is one of the leading causes of death in Canada,[1] and its incidence in Nova Scotia is higher than almost any other province.[1] Dysfunction of the innermost layer of blood vessels, the vascular endothelial cells,[2] can lead to the development of CVD.[3] A hallmark of endothelial dysfunction is reduced vasodilation (i.e. relaxation) of the adjacent vascular smooth muscle (VSM) cells due to reduced nitric oxide (NO) availability or production. Nitric oxide is an important vasodilatory chemical produced by endothelial nitric oxide synthase (eNOS) in the vascular endothelium that regulates resting blood flow and oxygen delivery.[4] Hyperemia (i.e. elevated blood flow) during exercise has also been shown to be partially regulated by NO.[5,6] Reactive oxygen species (ROS), produced in excess as a result of disease and smoking, or naturally during aerobic metabolism and exercise, can degrade NO into toxic peroxynitrite,[3] leading to further oxidative stress and endothelial dysfunction. However, antioxidants can inhibit oxidative stress, and reduce the formation and actions of ROS.[3]

Virgin coconut oil (VCO) has been steadily growing in popularity as a functional food oil,[7] due to its relatively high content of polyphenols.[8] Polyphenols, sometimes referred to as antioxidants, are a diverse group of compounds that upregulate the activity of various antioxidant enzymes.[9–15] Unlike traditional coconut oil, copra oil (CO), VCO is produced without use of a refining and bleaching process, and results in a pure oil that retains most of its polyphenol content.[8] When compared to CO, the increased polyphenol fraction of VCO has a superior ability to reduce lipid oxidation,[16,17] improve the lipid profile, [16] and upregulate antioxidant enzymes [18] in rats.

Along with the above mentioned benefits in animal studies,[19,20] VCO has been shown to improve cognitive function,[21] reduce the incidence of oxidative stress-induced hepatic injury,[22] diminish corticosterone and serum cholesterol levels,[23] and increase tissue and plasma antioxidant status.[24–27] It is conceivable that an increase in antioxidant status resulting from VCO ingestion in humans may lead to a reduction in ROS and a subsequent increase in NO bioavailability. Indeed, a focus of several animal studies is the potential of VCO to improve vascular function due to its significant polyphenol content.[16,28–31] VCO increases NO bioavailability and vascular reactivity in rats,[28] and it may also attenuate negative effects of pro-oxidative oils by reducing vasoconstriction,[28] lipid oxidation,[32] and blood pressure.[32]

Although the benefits of VCO in animal models is well studied, its effects on vascular health in humans are unclear. To date, human reports suggest various beneficial health effects of VCO including: a reduction in waist circumference (4 weeks × 30 ml VCO·day^{-1}),[33] an increase in healthy high-density lipoprotein cholesterol (4 weeks × 30 ml VCO·day^{-1}),[34] and even an improvement in the quality of life in breast cancer patients (20 ml VCO·day^{-1} during chemotherapy).[35] However, whether VCO improves function of the vascular endothelium in humans has not been determined. Furthermore, whether VCO enhances exercise-mediated hyperemia has never been investigated in animals or humans. Since 30 ml VCO·day^{-1} for 4 weeks is a dosage and duration deemed safe for human consumption [33] and sufficient to elicit a positive change in anthropometric [33] and physiological [34] parameters, this dosage and duration was chosen for the present study.

The objectives of the current study were to determine if a four-week ingestion of VCO (30 ml·day^{-1}) in young, healthy adults could enhance popliteal artery (PA) endothelial-dependent dilation (i.e. NO bioavailability) and augment the blood flow response induced by a single, brief

bout (10-min) of moderate-intensity cycling exercise. Endothelial-dependent dilation of the PA was evaluated via the Doppler ultrasound flow-mediated dilation (FMD) test. Results from the FMD test serve as a surrogate marker for endothelium-derived NO bioavailability in humans.[36] A final important objective of the current study was to uncover information that may change opinions on VCO use and modify its Health Canada classification to a food or natural health product.

It was hypothesized that short-term VCO ingestion in young, healthy adults would: 1) produce a greater PA FMD response, 2) augment the PA blood flow response to a brief bout of moderate-intensity cycling exercise and 3) increase plasma total antioxidant capacity (TAC).

Chapter 2: Review of Literature

2.1 – Overview of Vascular Structure and Function

2.1.1 – The Cardiovascular System

The cardiovascular (CV) system consists of a vast network of vessels that transport blood throughout the body.[37] The heart receives oxygenated blood from the pulmonary system and pumps it through the systemic conduit arteries. The conduit arteries, in turn, feed and branch into smaller arteries, arterioles and capillaries to exchange gases and nutrients to the organs.[37] This process is especially important for the delivery of oxygen and nutrients to active skeletal muscles during exercise. Once the exchange of nutrients is complete, deoxygenated blood is transported back to the heart through the venules and veins, and finally to the lungs for re-oxygenation.[37]

Arterioles have the greatest capacity to constrict and dilate, allowing them to rapidly adjust total peripheral resistance (i.e. opposing force against blood flow) and assist with the redirection of cardiac output to regions with the greatest metabolic demand (i.e. skeletal muscles during exercise).[38] The maintenance of total peripheral resistance allows arteries to control the driving pressure from the heart (i.e. mean arterial pressure) and ensure adequate delivery of essential nutrients to the body.[39] Furthermore, arteries gradually convert high-pressure pulsatile flow from the heart to lower-pressure steady flow to prevent damage to the smaller vessels.[39] In order to withstand these greater pressures and reduce pulsatile flow, arteries are composed of thick layers of VSM and a high degree of collagen and elastin to easily change diameter when needed.[40] Expansion of the arteries by the forceful pumping of the heart allows them to store the transferred energy (the passive elastic recoil) to maintain continuous blood flow. Passive elastic

recoil of the arteries is important because it does not require much energy and helps promote continuous laminar (i.e. streamline) flow throughout the CV system.[40]

2.1.2 – Layers of the Artery

Arteries and arterioles are composed of three layers, which all serve an important purpose (Figure 2.1). The outer layer, the tunica adventitia, contains sympathetic nerve innervation and the vascular blood supply (i.e. vaso vasorum). This layer is also composed of large amounts of collagen that protect and reinforce the blood vessel. The middle layer, the tunica media, is composed primarily of VSM cells, which are encased in a matrix of collagen, elastin and glycoproteins.[2] The primary function of the tunica media is to continuously maintain vascular tone (i.e. state of contractile tension in the vessel walls) and facilitate vasodilation and vasoconstriction.[2] The innermost layer of the artery is the tunica intima. The tunica intima is composed of a single layer of endothelial cells and a basement membrane. This layer serves as a selectively permeable barrier between the flowing blood in the lumen and the underlying tissues.[2]

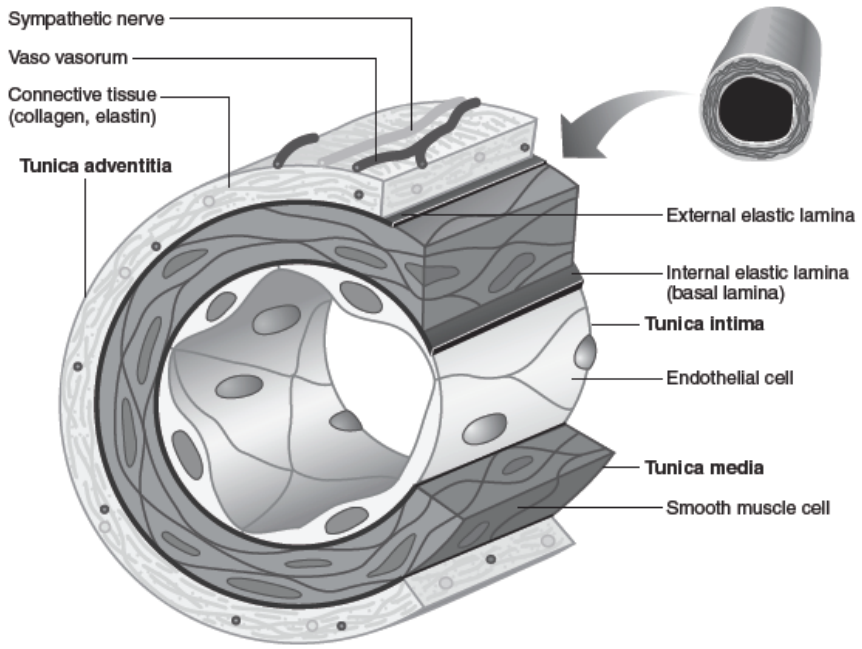

Figure 2.1: Cross-sectional representation of an artery, displaying the three main layers and the components of these layers.[2]

2.1.3 – Arterial Vasoconstriction and Vasodilation

As mentioned previously, the arteries help maintain systemic pressure by continuously contracting and relaxing as needed.[38] Vasoconstriction and vasodilation of the artery and arterioles are controlled both locally and extrinsically.[39] Locally, the vascular tone, or degree of contraction, is regulated by perfusion pressure (i.e. mean arterial pressure – venous pressure) and the chemical mediators produced in the endothelium. Perfusion pressure in the arterioles regulates the blood flow to the tissues.[39] If perfusion pressure increases, the response is contraction of the VSM layer to maintain a consistent quantity of blood flowing to the smaller vessels. This process is referred to as myogenic autoregulation and it is independent of the endothelium [39] (Figure 2.2).

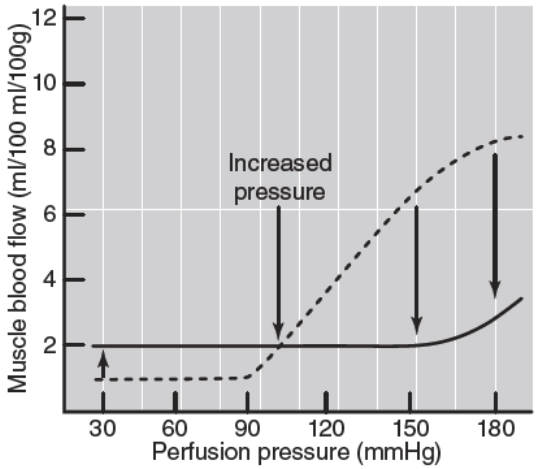

Figure 2.2: Myogenic autoregulation. Increasing perfusion pressure temporarily causes a corresponding change in arteriolar constriction to maintain continuous muscle blood flow.[39]

Chemical mediators of vasodilation (i.e. NO and prostacyclin) and mediators of vasoconstriction (i.e. endothelin-1) produced in the endothelium work together and maintain vascular tone and regulate blood flow distribution throughout the vascular system.[41] The specific roles of these mediators are explained below (section 2.2.1).

Extrinsically, vasoconstriction of the artery is influenced by the medullary CV control centers, hormones, baroreceptors, chemoreceptors, and the renin-angiotensin-aldosterone system.[39]

The medullary CV control center primarily elicits sympathetic stimulation to the vasculature, eliciting vasoconstriction.[39] Inhibition of sympathetic stimulation results in an opposing effect. Contraction of the VSM occurs when norepinephrine is released from the sympathetic nerve endings in the tunica adventitia, and binds to the α_1-adrenergic receptors present on the VSM [39] (Figure 2.3). Sympathetic stimulation is inhibited when norepinephrine binds to the α_2-adrenergic receptor on the sympathetic nerve. This binding acts as a feedback mechanism to decrease the release of norepinephrine [39] (Figure 2.3).

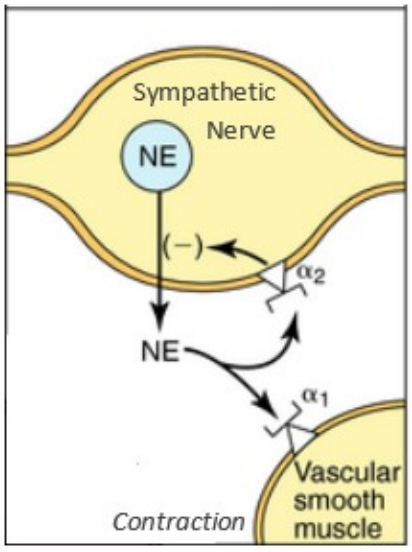

Figure 2.3: NE-mediated feedback mechanism to regulate sympathetic stimulation. Sympathetically-mediated contraction through NE binding to α_1-adrenergic receptors present on the VSM. Sympathetic stimulation is inhibited when NE binds to α_2-adrenergic receptors on the nerve. NE, norepinephrine; α_1, alpha-1-adrenergic receptors; α_2, alpha-2-adrenergic receptors.[42]

Additionally, epinephrine and norepinephrine are released from the adrenal medulla and transported through the circulation. These catecholamines are released in response to feedback

from receptors in the body, such as baroreceptors (i.e. pressure receptors) and chemoreceptors (i.e. detect changes in blood gases).[39] Both catecholamines bind to the α_1-adrenergic receptors on the VSM, eliciting vasoconstriction.[39] However, epinephrine and norepinephrine can also stimulate a vasodilatory response.[39] The majority of epinephrine is located within the plasma, therefore it binds to β_2-receptors located on the endothelial cells lining the lumen, resulting in vasodilation.[39] Norepinephrine can also bind to β_2-receptors. However, β_2-receptors have a significantly greater affinity for epinephrine than for norepinephrine.[43]

The renin-angiotensin aldosterone system triggers the release of other hormones to control blood pressure.[39] In response to a drop in blood pressure, renin is released from the kidney to convert angiotensinogen to angiotensin I. Angiotensin I is then converted to angiotensin II (a powerful vasoconstrictor) by the angiotensin converting enzyme. Angiotensin II increases blood pressure by increasing vascular resistance and mediating the release of aldosterone, a hormone which stimulates fluid retention and increases blood volume.[39]

2.1.4 – Vascular Smooth Muscle Contraction

Vascular smooth muscle cells are small, spindle-shaped, 'excitable' cells that conduct an action potential.[2] The VSM cells are interconnected via gap junctions that allow the action potential to spread across all VSM cells for a coordinated contraction (Figure 2.4). Similar to skeletal muscle, the contractile proteins of VSM are myosin and actin.[2] Actin is anchored to the interior and surface of the cell by dense bodies and dense bands, respectively. The dense bodies are connected via intermediate filaments and form a cytoskeleton framework across the VSM.[2] Myosin filaments, the other contractile proteins, are woven between the actin filaments (Figure 2.4).

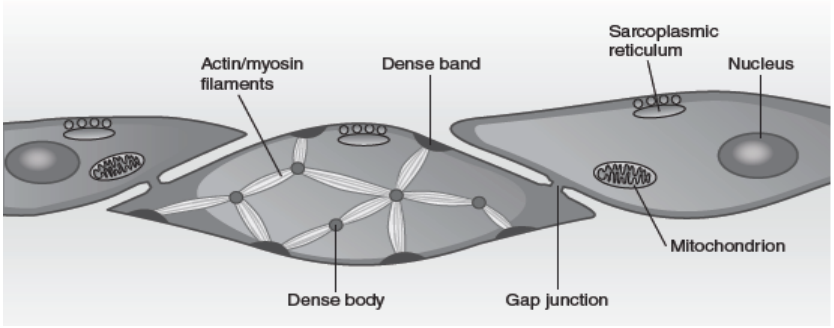

Figure 2.4: Organization of the contractile proteins of smooth muscle.[2]

When there is an influx of calcium (Ca^{2+}) in the cell, Ca^{2+} will bind to calmodulin, activating the enzyme myosin light-chain kinase [2] (Figure 2.5). Myosin light-chain kinase phosphorylates the light chains on the myosin head and allows the myosin head to attach with actin, causing contraction of the VSM. When Ca^{2+} levels deplete (i.e. re-uptake into the sarcoplasmic reticulum), myosin light-chain phosphatase removes the phosphate group and the actin and myosin filaments separate, resulting in relaxation of the VSM [2] (Figure 2.5).

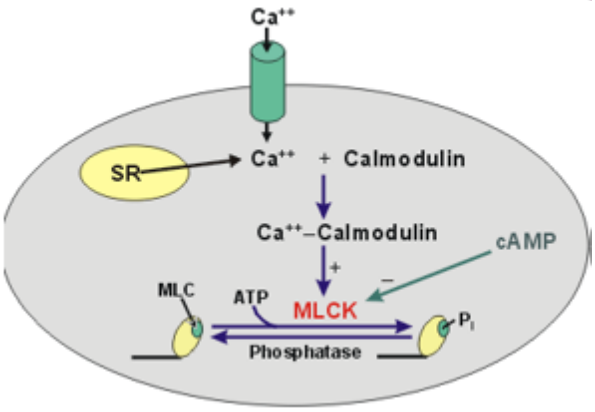

Figure 2.5: Contraction of the VSM with MLCK. An influx of Ca^{2+} into the VSM cell, through Ca^{2+} channels or from the SR, binds with calmodulin and activates MLCK. MLCK facilitates the binding of actin to myosin heads resulting in contraction of the VSM. Ca^{2+} reuptake into the SR reverses this process, and allows the VSM to relax. SR, sarcoplasmic reticulum; Ca^{++}, calcium; MLC, myosin light chain; ATP, adenosine triphosphate; MLCK, myosin light-chain kinase; cAMP, cyclic adenosine monophosphate; P_i, inorganic phosphate.

2.2 – Roles of the Arterial Vascular Endothelium

Aside from regulating vascular tone, the vasoactive chemicals produced in the endothelium also possess the ability to mediate the release of anticlotting and pro-clotting factors (Figure 2.6). Mediators of vasodilation have an inhibitory effect on platelet accumulation (i.e. pro-clotting factors) and can maintain blood fluidity.[2,41] The endothelium also plays an important role in the defense against pathogens (Figure 2.6). In response to endothelial injury, adhesion molecules [i.e. platelet-selectin (P-selectin), endothelial-selectin (E-selectin), intracellular adhesion molecules (ICAM) and vascular cell adhesion molecules (VCAM)] are released from vascular endothelial cells, bind leukocytes to the site of injury and create an inflammatory barrier.[2,41] The last major role of the endothelium involves the initiation of new blood vessel formation (i.e. angiogenesis; Figure 2.6). Endothelial cells can rapidly divide when there is need for new blood vessels throughout the body, such as following ischemic injury or during exercise.[2,41]

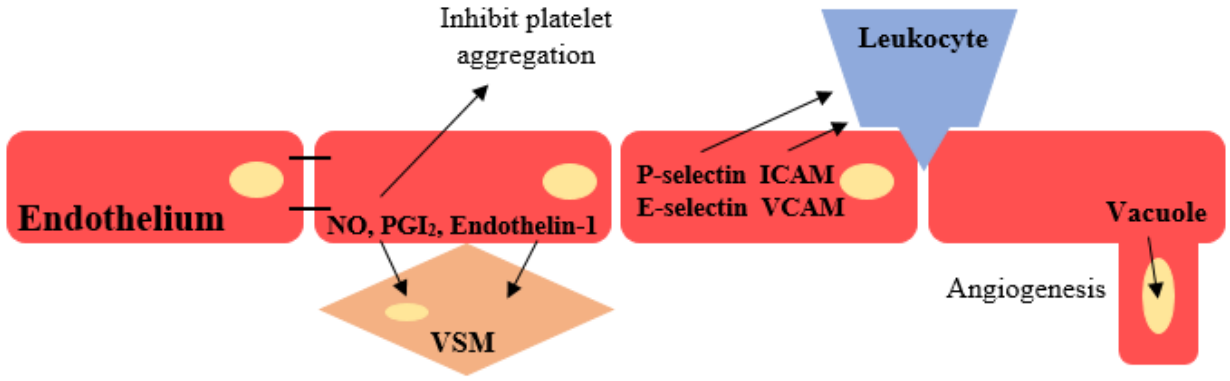

Figure 2.6: Roles of the endothelium including mediating the release of clotting factors (e.g. inhibition of platelet aggregation), defense against pathogens (e.g. leukocytes) and angiogenesis. NO, nitric oxide; PGI_2, prostacyclin; VSM, vascular smooth muscle; P-selectin, platelet-selectin; E-selectin, endothelial-selectin; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule.

2.2.1 – Vasoactive Compounds of the Endothelium

Many chemical mediators are released from the endothelium that play a role in maintaining vascular tone and platelet accumulation. The most important vasoconstrictor produced in the endothelium is endothelin-1.[2] Endothelin-1 is a very potent vasoconstrictor with a low rate of formation that can sustain vasoconstriction for approximately 2-3 hours.[2] Binding of endothelin-1 to its receptor promotes the formation of vasodilatory chemicals such as NO, which can inhibit its action. Therefore, in a healthy artery, a balance between vasoconstriction and vasodilation is maintained. Two important vasodilatory substances released from the endothelium are prostacyclin and endothelium-derived hyperpolarizing factor.[2] Prostacyclin and endothelium-derived hyperpolarizing factor are both activated when agonists (i.e. acetylcholine, bradykinin, substance P, etc.) bind to specific endothelial receptors. Through this activation, prostacyclin and endothelium-derived hyperpolarizing factor contribute to VSM relaxation. Although these two chemicals aid in VSM relaxation, the most potent vasodilator produced in the endothelium is NO.[2]

2.2.2 – Action of Nitric Oxide

Nitric oxide is produced in the vascular endothelial cells by eNOS (Figure 2.7). Endothelial nitric oxide synthase is regulated by mechanisms such as intracellular calcium (Ca^{2+}) concentrations and shear stress.[2,44] Certain chemicals such as acetylcholine, histamine, bradykinin, adenosine triphosphate, thrombin and substance P act as agonists for NO production.[2,44] When these chemicals bind to specific receptor-operated channels, Ca^{2+} is released from the endoplasmic reticulum, increasing Ca^{2+} concentrations of the endothelial cell.[2] Intracellular Ca^{2+} then binds with calmodulin to form Ca^{2+}-calmodulin complexes, which mediate the conversion of L-arginine to NO and L-citrulline by eNOS [2,45,46] (Figure 2.7).

Endothelial nitric oxide synthase can also be activated by shear stress, or the force of blood flowing over the vascular endothelial cells [44] (Figure 2.7). Shear stress stimulates proteins, which enhance the catalytic activity of eNOS,[47] even at resting Ca^{2+} concentrations.[44] In situations where blood flow is enhanced, such as during exercise, shear stress is greater and stimulates a larger degree of NO production.[2]

Once NO is produced, it diffuses into the VSM through myoregulatory junctions [2] (Figure 2.7). Once in the VSM, NO activates the enzyme guanylate cyclase.[2] Guanylate cyclase catalyzes the conversion of guanosine triphosphate into cyclic-guanosine monophosphate (cGMP; Figure 2.7). Once produced, cGMP activates protein kinase G, an enzyme with multiple functions.[2] Protein kinase G phosphorylates Ca^{2+}-ATPase pumps to remove Ca^{2+} from the VSM and promotes Ca^{2+} uptake into the endoplasmic reticulum. Furthermore, it inhibits Ca^{2+} channels from allowing extracellular Ca^{2+} to enter into the VSM cell, and it activates outward rectifying potassium channels, which removes intracellular potassium and inhibits action potential formation (Figure 2.7). These actions hyperpolarize the VSM cell and allow the VSM to relax and the artery to dilate.[2] It should also be noted that the activity of cGMP is modulated by phosphodiesterase-type 5. Phosphodiesterase-type 5 dissociates cGMP into 5-guanosine monophosphate, inhibiting its function, and facilitating constriction of the VSM.[2]

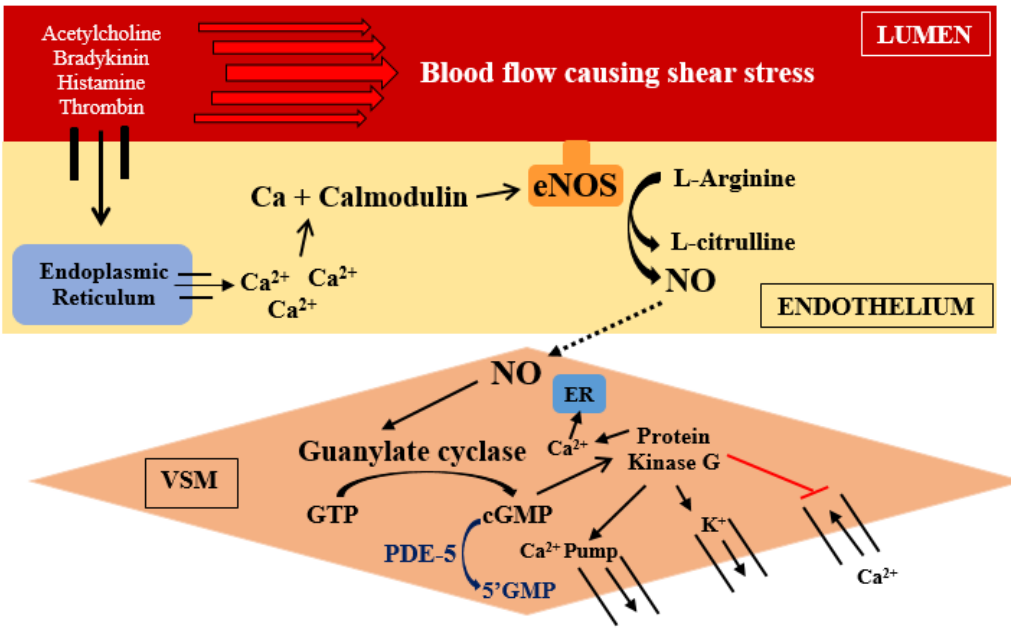

Figure 2.7: NO production and mechanism of action. Red line indicated inhibitory process. Blue reaction represents dissociation of second messenger. Ca^{2+}, calcium; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; VSM, vascular smooth muscle; GTP, guanosine triphosphate; cGMP, cyclic-guanosine monophosphate; ER, endoplasmic reticulum; K^{+}, potassium; PDE-5, phosphodiesterase-type 5; 5'GMP, 5-guanosine monophosphate; Ca^{2+}Pump, calcium-adenosine triphosphatase pump.

Nitric oxide has many other important functions aside from its vasodilatory properties. Nitric oxide prevents platelet accumulation and adhesion, it limits oxidation of low-density lipoprotein-cholesterol (LDL-C), and it inhibits proliferation of VSM cells.[2,45,48] Nitric oxide-mediated pathways can also decrease the expression of pro-inflammatory genes that advance atherosclerosis,[45,48,49] and modulate neuronal activity, metabolism and immune responses.[50]

2.3 – Exercise-Mediated Hyperemia

Dynamic exercise augments the metabolic demand in human skeletal muscle, and blood flow must increase to supply adequate nutrients and oxygen to the active tissue.[51] Neuronal, metabolic, endothelium-derived, and mechanical factors have been implicated in the control of

exercise-mediated hyperemia.[52] The flow of blood throughout the system, relies on perfusion pressure (i.e. mean arterial pressure – central venous pressure) and total peripheral resistance.[53] The pressure in the system increases gradually, however the main factor that influences blood flow is vascular resistance.[52] Vascular resistance can be influenced by neural and local control mechanisms. Sympathetic activation to the heart and blood vessels increases cardiac output (i.e., product of heart rate and stroke volume) and constricts blood vessels in less active regions of the body to help redirect cardiac output to active skeletal muscles.[52] Local vasodilatory control and the skeletal-muscle pump (description below) help mediate exercise hyperemia to active muscles (Figure 2.8).

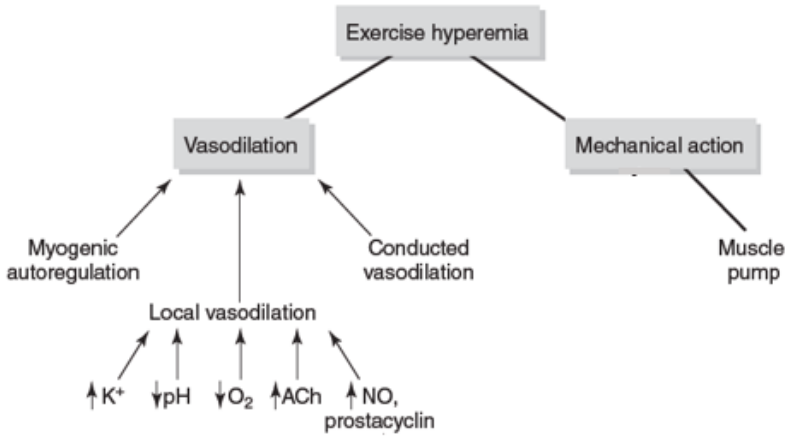

Figure 2.8: Schematic representation of factors facilitating exercise hyperemia including mediators of vasodilation and mechanical action. K^+, potassium; pH, potential of hydrogen; O_2, oxygen; ACh, acetylcholine; NO, nitric oxide.[54]

Local control of vasodilation occurs by an influx of metabolites from the actively contracting skeletal muscles.[52] These metabolites diffuse into the interstitial fluid to the resistance arterioles and induce vasodilation and capillary formation.[52] An influx of potassium, acetylcholine, NO and prostacyclin, as well as a decrease in pH and oxygen can induce vasodilation [53] (Figure 2.8).

It has been well-documented that NO plays a role in exercise-mediated hyperemia of the lower limb arteries.[55–58] Radegran & Saltin [6] assessed exercise-mediated hyperemia of the femoral artery in healthy men (25 ± 1 years) following one-legged dynamic knee extensor exercise with an infusion of L-N^G-monomethyl arginine (L-NMMA). L-NMMA competes with L-arginine, blocks the active site on eNOS and prevents NO production.[59] Doppler ultrasound was used to measure leg blood flow of the femoral artery. Inhibition of eNOS by L-NMMA caused a strong reduction in femoral artery blood flow during rest and post-exercise recovery.[58]

Similarly, Mortensen et al.[55] assessed the effect of three inhibitory drugs of vasodilation on exercise-mediated hyperemia of the femoral artery following one-legged knee-extensor exercise (20 Watts) in healthy moderately trained male subjects (24 ± 4 years). Trial 1 involved the infusion of indomethacin (INDO; prostacyclin [PGI_2] inhibitor), trial 2 involved the infusion of a combination of INDO and L-NMMA, and trial 3 involved a combination of INDO, L-NMMA and tetraethylammonium chloride (TEA; cyclooxygenase inhibitor). Leg blood flow was monitored using Doppler ultrasound. The PGI_2 inhibitor (INDO) alone did not elicit a significant reduction in leg blood flow. However, a significant reduction following trial 2 (INDO + L-NMMA) and trial 3 (INDO + L-NMMA + TEA) was observed. Additionally, there were no significant differences between trials 2 and 3, indicating that NO may have been a primary contributor to exercise-mediated hyperemia.[55] Mortensen et al.[56] repeated the same study design as previous,[55] but replaced TEA with theophylline (a P1 adenosine receptor inhibitor). Adenosine can induce vasodilation (see below). Blood samples were also collected to determine the plasma concentrations of NO and PGI_2 following adenosine infusion. Adenosine receptor blockade alone, or inhibition of PGI_2 (INDO) and NO (L-NMMA) synthesis lowered exercise-hyperemia similarly. However, when combined (i.e. theophylline + L-NMMA + INDO) the drugs did not

have an additive effect, and the researchers concluded that the action of adenosine was achieved by stimulating PGI_2 and NO synthesis.[56] Therefore, enhancing NO synthesis may augment the hyperemic response to exercise.

Adenosine and histamine can also bind to receptors on the VSM and vascular endothelium to aid in vasodilation [53] (Figure 2.9). Adenosine can bind to A2 receptors on the endothelial cells to induce vasodilation, or to the A1 receptors on the sympathetic nerve endings to inhibit norepinephrine release and the resulting vasoconstriction. Histamine can bind to H1 receptors on the endothelial cells and trigger vasodilation, or to H2 receptors on the VSM and promote Ca^{2+} uptake into the endoplasmic reticulum.[53]

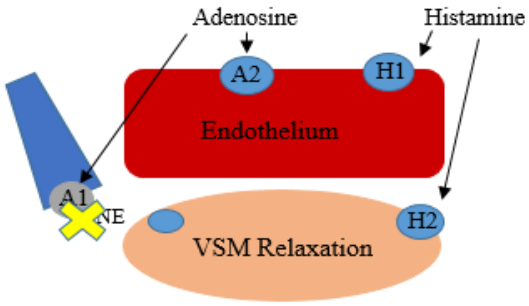

Figure 2.9: Adenosine binding to A1 and A2 receptors results in vasoconstriction and vasodilation, respectively. Histamine binding to H1 and H2 receptors results in vasodilation and Ca^{2+}, respectively.

Conducted vasodilation also aids in exercise hyperemia.[53] Hyperpolarization can be conducted through endothelial/smooth muscle cells through gap junctions, spreading dilation to different levels of the arterial system [53] (Figure 2.10).

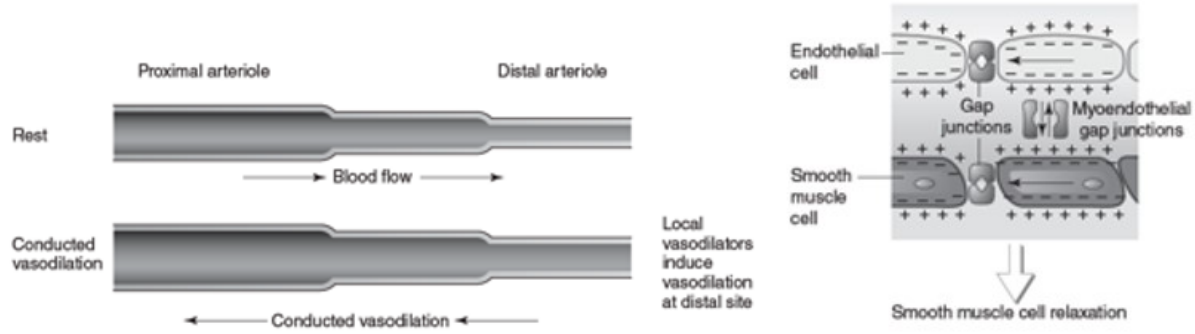

Figure 2.10: Conducted vasodilation through hyperpolarization across the gap junctions, down the arteriole.[54]

The muscle pump also aids in the transport of blood back to the heart during rhythmic exercise. With each contraction of a skeletal muscle, blood is forced through the venous system back to the heart, and the one-way venous values prevent backflow.[53] This induces a negative venous pressure (i.e. suction) that 'pulls' blood through from the arterial system. Immediately post-contraction, the muscle pump works with vasodilation to increase blood flow (net hyperemic response).[53]

2.4 – Consequences of Oxidative Stress

Oxidative stress refers to the relative overproduction of ROS, or more specifically, when the pro-oxidant enzymes overwhelm the antioxidant defense systems.[4] Oxidative stress can occur for a variety of reasons including CVD, or its primary risk factors such as a high-fat diet, smoking, aging, hypertension,[9] diabetes mellitus,[60] hypercholesterolemia,[61] or atherosclerosis.[62] These risk factors lead to dramatic increases in ROS concentrations in the vascular endothelium. Subgroups of ROS produced in the endothelium include free oxygen radicals, oxygen ions, and peroxides.[4,63,64]

2.4.1 – ROS-Producing Enzyme Systems

There are four main enzyme systems that produce ROS in the vessel: nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, enzymes of the mitochondrial electron transport chain, and dysfunctional eNOS.[4] NADPH oxidase is a multicomponent enzyme present in the wall of blood vessels.[65,66] The primary function of NADPH oxidase is to produce superoxide, a ROS that can rapidly degrade NO. Several studies have shown an upregulation of NADPH oxidase in animal and human models of CVD and atherosclerosis.[62,65,66] Xanthine oxidase is another ROS-producing enzyme that donates electrons to molecular oxygen, producing superoxide and hydrogen peroxide.[4] It has been shown that increased plasma cholesterol concentrations may stimulate the release of xanthine oxidase from the liver into the circulation.[67] Inhibition of xanthine oxidase, using oxypurinol, improves endothelial-dependent vascular relaxation in response to acetylcholine, indicating that xanthine oxidase may contribute to endothelial dysfunction.[68,69] However, the importance of xanthine oxidase in endothelial dysfunction is less well understood than that of NADPH oxidases.[4]

Enzymes of the mitochondrial respiratory chain are also capable of producing ROS. It has been shown that 1% of the oxygen consumed in the respiratory chain is reduced (i.e. gains an electron) to form superoxide radicals.[70] Superoxide can be produced in two complexes of the electron transport chain: complex I (NADH dehydrogenase) and in complex III (ubiquinone-cytochrome b-c1 region).[70] The production of superoxide is usually counteracted by the activity of manganese superoxide dismutase (Mn-SOD), which rapidly converts superoxide into hydrogen peroxide.[4,70] However, there is evidence that certain diseases of the CV system can result in mitochondrial dysfunction [71] and Mn-SOD deficiency.[72] Therefore, the superoxide produced can be harmful and may degrade NO.

The final ROS-producing enzyme system is dysfunctional eNOS. If eNOS becomes dysfunctional, its ability to produce NO is limited, and it may stop the production of NO altogether.[73] In order to produce NO, eNOS must complete two cycles, in which it binds, and activates oxygen twice. The first cycle involves the conversion of L-arginine to N-hydroxyl-L-arginine, and the second cycle involves the conversion of N-hydroxyl-L-arginine to citrulline and NO.[73,74] If eNOS does not carefully control the quantity and tempo of electron transfer in these two cycles, free oxygen can be released (i.e. superoxide = free oxygen radical). Low intracellular L-arginine can predispose eNOS to generate superoxide by uncoupling (i.e. not allowing or disconnecting) oxygen reduction at the flavin binding site of eNOS.[73,75] Prolonged Ca^{2+} influx from glutamate receptor stimulation has also been demonstrated to promote superoxide production by NOS present in the neurons (i.e. neuronal NOS).[76] Furthermore, auto-oxidation (i.e. oxidation incurred by self-produced ROS) at the reductase domains of eNOS can occur following the production of superoxide radicals.[73] NADPH oxidase likely contributes to oxidation of eNOS as well due to its significant contribution to intracellular superoxide production.[77] Auto-oxidation of the reductase domains limits the two-cycle electron reduction needed to generate NO. However, it still allows one-electron transfer to generate superoxide radicals. Therefore, with prolonged auto-oxidation, eNOS can become completely dysfunctional.[73] Evidence for eNOS uncoupling has been reported in patients with varying degrees of endothelial dysfunction.[78–80]

It has also been suggested that downregulation of the asymmetric dimethylarginine (ADMA)-degrading enzymes by superoxide can contribute to eNOS uncoupling.[81] ADMA competes with L-arginine and inhibits eNOS activity.[82] The enzyme responsible for the breakdown of ADMA is dimethylarginine dimethylaminohydrolase (DDAH). Factors associated

with elevated levels of ROS such as CVD, aging, hypertension, and smoking, have been linked to the inhibition of DDAH. Inhibition of DDAH leads to elevated ADMA concentrations that compete with L-arginine, and inhibit the ability of eNOS to produce NO. This inhibition of eNOS activity can also contribute to its uncoupling and enhance the production of superoxide radicals.[83]

2.4.2 – NO, Superoxide and Peroxynitrite

The oxidative stress produced by ROS eventually leads to dysfunction of the vascular endothelial cells, primarily through reducing the half-life of NO [84] (Figure 2.11). When produced in excess (i.e. aerobic metabolism) superoxide rapidly pairs with NO to form the highly reactive and highly toxic peroxynitrite.[85,86] Peroxynitrite is a strong oxidant and is stable as an anion at alkaline pH, making it particularly toxic.[85] This stability allows peroxynitrite to diffuse through a cell and locate a target for oxidation, creating oxidative compounds such as carbonyl, salicylate and hydroxyl radicals through many complex mechanisms.[85]

Furthermore, as mentioned previously, persisting oxidative stress can also render eNOS dysfunctional (i.e. auto-oxidation).[73] Oxidation of the reductase domains by peroxynitrite can inhibit regular eNOS activity and promote the formation of superoxide rather than NO (Figure 2.11).[48,49]

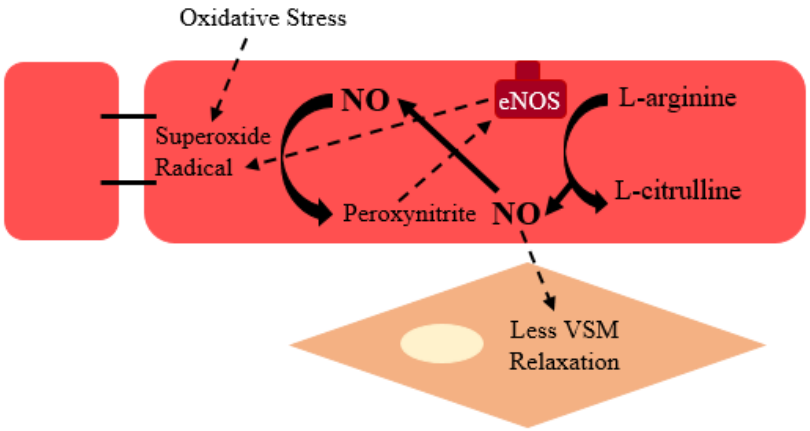

Figure 2.11: Rapid NO conversion into peroxynitrite by superoxide. Once produced, peroxynitrite can oxidize eNOS (i.e. auto-oxidation), causing eNOS to produce superoxide, rather than NO. NO, nitric oxide; eNOS, endothelial nitric oxide synthase.

2.4.3 – LDL Oxidation

Another way that ROS can elicit damage to the endothelium is through oxidative modification of LDL (Figure 2.12). Oxidative modification of LDL by ROS is an important preliminary step in the development of atherosclerosis.[87] A diet high in saturated fat leads to accumulation of LDL in the circulation, increasing the likelihood that LDL becomes trapped beneath the vascular endothelial cells.[88] Once LDL molecules traverse the sub-endothelial space of the artery, the LDL molecules are subject to oxidation by ROS present in the endothelial cells. Recently it has been shown that small dense LDL molecules possess even greater atherogenic potential, and presence of these molecules can indicate a higher risk of developing atherosclerotic disease.[89]

Oxidation (also referred to as peroxidation) of the LDL molecules primarily occurs through transformation of the apolipoprotein B-100 protein.[88] The apolipoprotein B-100 lysine groups are modified so that the net negative charge of the lipoprotein particle increases.[90] Once transformed, the LDL molecules act as a chemoattractant for monocytes and macrophages.[91]

When too many LDL molecules bind to the macrophages, the macrophages become saturated and undergo transformation into cholesterol ester-laden foam cells, a characteristic stage of atherosclerotic disease.[3] Furthermore, now that this area has been identified as a site of injury, additional macrophages and monocytes will migrate into the cell and can exacerbate the effects. Eventually, with prolonged accumulation of oxidized LDL and immune defense molecules, the foam cells can become necrotic and lead to severe endothelial injury, dysfunction, and death.[88]

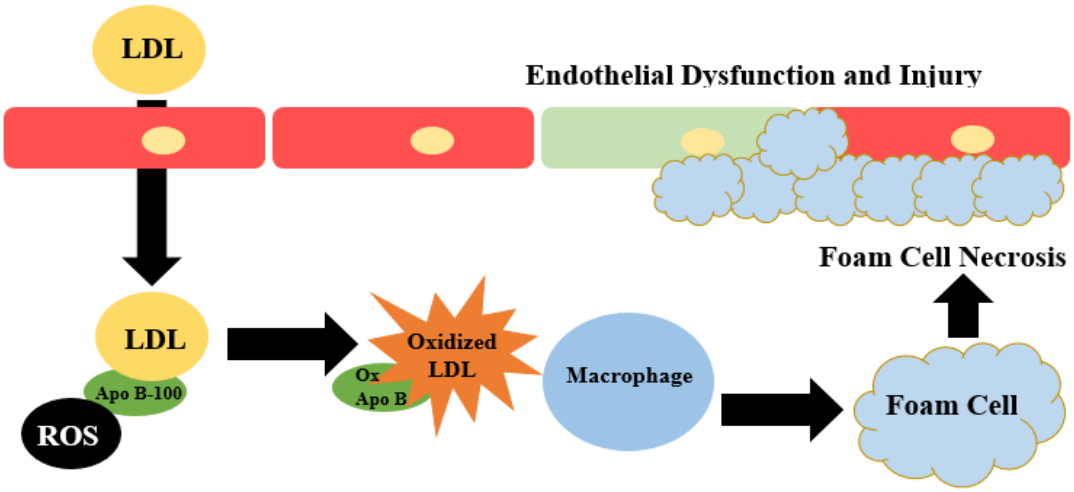

Figure 2.12: A high-fat diet can enhance sub-endothelial infiltration of LDL molecules, leading to the formation of damaging foam cells by ROS. Eventually, foams cells can become necrotic and cause endothelial injury and dysfunction. LDL, low-density lipoprotein; ROS, reactive oxygen species; Apo B-100, apolipoprotein B-100; Ox Apo B, oxidized apolipoprotein B.[88]

However, this process can only occur when there are enough oxidants to overwhelm the antioxidant defenses. Antioxidants have been shown to reduce the concentration of ROS in the vessel walls, thus reducing the concentration of LDL molecules modified by oxidation.[4,88] Reducing the content of oxidized LDL molecules will potentially improve endothelial function, increase NO availability and production, and therefore increase vasodilation.

2.5 – Antioxidant Defense Systems

Antioxidant enzymes that have been shown to protect against oxidative stress include: superoxide dismutase, glutathione peroxidase, catalase, and paraoxonase. Superoxide dismutase converts two molecules of superoxide into oxygen and hydrogen peroxide molecules, neutralizing its pro-oxidative effects.[92] Two primary forms of superoxide dismutase important in CV protection are copper/zinc superoxide dismutase (Cu/Zn-SOD), located in the cytosol and extracellularly, and Mn-SOD, located in the mitochondria.[4] As mentioned previously, Mn-SOD located in the mitochondria is the first line of defense against superoxide production by the mitochondrial respiratory chain.[4,70] However, Cu/Zn-SOD has also been demonstrated to be important in CV protection. This form of SOD has been demonstrated to inhibit superoxide and peroxynitrite production in murine peritoneal macrophages [93] and decrease lipid peroxidation in rodent models.[94] Other reports suggest that injection of lysosome-entrapped bovine [95] or human [96] Cu/Zn-SOD can reduce blood pressure in hypertensive rats.

Although SOD is a power antioxidant, it can also have pro-oxidant properties. Hydrogen peroxide, a by-product of the reaction catalyzed by SOD is toxic to cells.[97] However, glutathione peroxidase and catalase are antioxidants that reduce hydrogen peroxide to water and oxygen.[4,92] Overexpression of catalase can delay the development of atherosclerosis, likely due to the upregulation of hydrogen peroxide break-down.[98]

A final important group of antioxidant defense enzymes are paraoxonases. Paraoxonases have been shown to reduce (i.e. donate an electron) and neutralize superoxide in human vascular endothelial cells, VSM cells, and fibroblasts.[99] Activity of paraoxonases may be upregulated by polyphenols.[24]

2.5.1 – Polyphenolic Antioxidants

Current evidence suggests that polyphenols possess some ability to prevent ROS damage. This is primarily supported by the negative correlation between consumption of polyphenol-rich foods or beverages (fruits, vegetables, red wine, green tea, etc.) with the incidence of CVD.[100–103] Although most polyphenols have been classified as mild antioxidants, some have been shown to have a significant ability to reduce the activity of NADPH oxidases, while others have been shown to stimulate anti-oxidative enzymes and eNOS.[9–14] Certain foods, and the polyphenolic antioxidant content of these foods, have begun to gain recognition as beneficial preventative measures against oxidative stress and cardiovascular disease.

2.5.2 – Measurement of Plasma Antioxidant Content

A useful tool to assess for total antioxidant content or capacity in foods or human fluids is the TAC enzyme-linked immunosorbent assay (ELISA). Total antioxidant capacity represents the overall antioxidant activity of the diet.[104] Quantitative determination of plasma TAC can be achieved through use of an anti-TAC antibody, and a TAC-horseradish peroxidase (HRP) conjugate. Plasma samples are incubated together with TAC-HRP conjugate and competitive binding for the antibody binding sites occurs. Methodological procedures for TAC detection are internally validated by the institution from which the assay is obtained. In the current study, the TAC ELISA was employed to measure plasma TAC (see Methods section 3.5.1).

2.6 – Virgin Coconut Oil

2.6.1 – Processing of VCO

Virgin coconut oil is a functional food oil typically produced from coconut milk, via wet processing.[7] The most common method of extracting coconut oil is the dry method which involves pressing, refining, bleaching and deodorizing (RBD) copra, resulting in the most

common form of coconut oil, copra oil (CO).[7] The primary issue with CO is that throughout the manufacturing process, a large portion of the antioxidant properties are lost. Virgin coconut oil on the other hand is produced without use of the RBD process, and results in a pure oil that retains most of its health benefits.[7] As such, many studies that have compared the effects of the two oils have found that the health benefits of VCO are far superior to its RBD-processed counterpart.[16,17,20,24,105,106]

2.6.2 – VCO and Polyphenols

Much of the antioxidant property of VCO can be attributed to its polyphenol content.[30] The phenolic content in VCO is much higher than CO because, as mentioned previously, VCO does not undergo the RBD process.[7] The polyphenol content of VCO has been estimated to be approximately 84 mg/100 g, and is higher than CO (64.4 mg), olive oil (75.63 mg) and sunflower oil (55.26 mg).[24] The primary phenolic acids present in VCO are ferulic acid and p-coumaric acid.[8] Ferulic acid possesses antioxidant and anti-inflammatory properties,[107] while p-coumaric acid also possesses antioxidant properties, and provides protection against the toxic effects of chemotherapy.[108] Other phenolic acids found in VCO, but not in CO, include vanillic acid, caffeic acid and syringic acid.[8] Caffeic acid can neutralize ROS,[109] likely due to its chemical structure that supports antioxidant activity (i.e. double bond conjugated with phenolic ring, and a carbonyl group).[110,111] Additionally, caffeic acid decreased the concentration of interleukin-1β, an inflammatory cytokine (i.e. promotes inflammation), by 40%.[112] Caffeic acid also increased the resistance of LDL to peroxidation and prevented the oxidative modification of apolipoprotein B-100, an important stage in oxidative modification of LDL.[113] The current literature investigating the effects of vanillic acid and syringic acid is sparse. However, vanillic acid may contribute to the stimulatory effect of red wine on human eNOS.[14]

Marina et al.[8] found very strong correlations between the total phenolic content of VCO and its scavenging and reducing power, indicating that the contribution of antioxidant activity in VCO was likely due to the phenolic compounds. Other studies also claim that polyphenols found in VCO can reduce the risk of CVD through scavenging ROS, inhibiting pro-oxidant enzymes, and reducing inflammation and platelet aggregation (i.e. accumulation).[30] Additionally, it has been suggested that polyphenols may indirectly reduce blood pressure,[28] improve vascular function,[30,31] and promote NO-mediated vascular relaxation.[28] Recently, it was demonstrated that the polyphenolic fraction of VCO was able to protect HCT-15 cells (i.e. colon epithelial cells) from pro-oxidant injury by restoring the activity of antioxidants such as glutathione peroxidase and catalase while concurrently decreasing lipid peroxidation.[114]

Polyphenol fractions from VCO have also been compared with those from other oils. The polyphenol fraction of VCO, ground nut oil, and CO was extracted, and the ability of the polyphenol fraction to inhibit carbonyl formation, a marker of oxidative stress and LDL oxidation, during *in vitro* copper-induced LDL oxidation was assessed. When compared to the other oils, the isolated polyphenol fraction from VCO demonstrated an enhanced ability to lower cholesterol and attenuate the oxidation of LDL molecules, both important risk factors for CVD. This was attributed to its relatively higher content of phenolic acids than the other oils [16,24] (Figure 2.13).

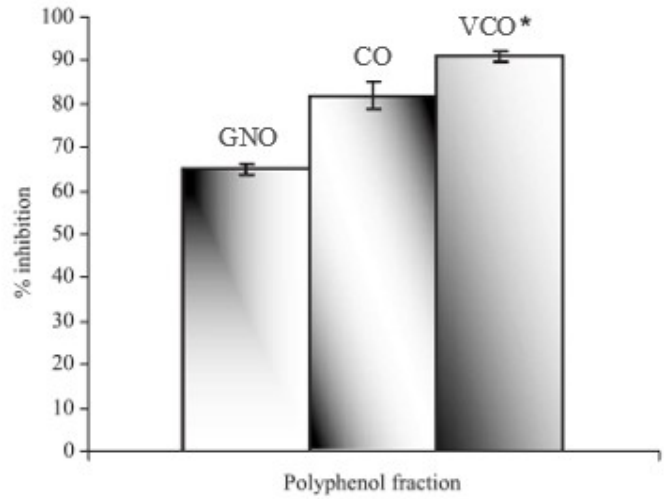

Figure 2.13: When compared to ground nut oil and CO, VCO was more effective at inhibiting carbonyl formation, a marker of oxidative stress and LDL oxidation. GNO, ground nut oil; CO, copra oil; VCO, virgin coconut oil. *, $p < 0.05$ vs. CO.[16]

It has not been demonstrated in human or animal models whether VCO can improve endothelial-independent vasodilation. However, studies have been conducted on the effects of certain polyphenols on sublingual NTG administration.[115–117] Hodgson et al.[117] studied the effects of polyphenol-rich black tea (5, 250ml cups per day, for 4 weeks) on endothelial-independent vasodilation in individuals with mild hyperlipidemia. It was found that the tea improved endothelial-independent vasodilation in these individuals.[117] Conversely, Djoussé [115] studied healthy non-smoking volunteers (32 ± 9 years) to assess whether a single high-fat meal with red wine, or with an isocaloric amount of Coca-Cola (3 $ml \cdot kg^{-1}$ body weight) affected the vasodilatory response to nitroglycerin. It was found that the vasodilator response to nitroglycerin was not affected by the high-fat meal or either beverage.[115]

Fusi & Sgaragli [116] assessed whether freeze-dried red wine could reverse nitrate tolerance commonly found in patients suffering from coronary artery disease, in rat aortic rings. It was found that the red wine polyphenols were capable of reversing tolerance to nitrates and that it

was potentially mediated by SOD.[116] Certain polyphenols that are present in red wine are also present in VCO (i.e. vanillic acid, p-coumaric acid, and caffeic acid).[14] Therefore, these conflicting results indicate that it was worthwhile to assess what the impact of VCO would be on endothelial-independent dilation of the VSM.

2.6.3 – VCO and Vitamin E

In addition to phenolic acids, VCO contains higher amounts of vitamin E than CO, olive oil, and sunflower oil.[24] Vitamin E has the ability to reduce the quantity of free-oxygen radicals by donating electrons and neutralizing their oxidative power.[118] A review compiled by Diaz et al.[119] concluded that *in vitro*, high doses (i.e. 100 IU to 250 IU) of vitamin E were able to block the oxidative modification of LDL cholesterol, decreasing their deposition in arterial walls. It was also concluded that vitamin E reduced monocyte adhesion to the endothelium and inhibited platelet activation, both of which are important atherosclerotic events. However, Duvall et al.[3] noted that in order to observe these CV benefits, vitamin E must be administered in larger doses (i.e. > 100 IU). Furthermore, extremely high doses of vitamin E (i.e. 400 IU to 800 IU) have been employed and significant reductions in CV event risk were either only present following 1.4-year supplementation,[120] or non-existent.[121] Numerous reviews concur with Duvall et al.[3] and state that although vitamin E has been believed to be a potent ROS-scavenger, large-scale randomized clinical trials have failed to demonstrate this.[4,122]

Despite this conclusion, when combined with phenolic compounds, as is the case in VCO, there appears to be promise in the proposed cardioprotective effects.[123] Upston et al.[123] found that overall, the effect of vitamin E alone on atherosclerotic events was very small or non-existent. However, it was noted that the balance between vitamin E and co-antioxidants (i.e.

phenolic acids) may exert a stronger effect on these events. Overall, it is clear that further research is required to fully understand the complexity of these antioxidant interactions.

2.7 – VCO Animal Studies

2.7.1 – VCO and Antioxidant Enzymes

The current evidence regarding ROS-scavenging ability of VCO is positive and abundant. Arunima & Rajamohan [24] published a report in which male Sprague-Dawley rats were fed a 12 g diet with 8% VCO, CO, olive oil, or sunflower oil for 45 days to assess the relative impact on endogenous antioxidant status and paraoxonase-1 activity, an antioxidant which reduces pro-oxidant superoxide molecules. It was found that when compared to CO, olive oil, or sunflower oil, dietary VCO was most effective at enhancing the activity of antioxidants including catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase. Arunima & Rajamohan [24] also found that the activity of paraoxonase-1 was significantly increased following VCO ingestion compared to the other oils. Upregulation of these antioxidants indicates enhanced superoxide and hydrogen peroxide breakdown,[97] reduced formation of peroxynitrite,[85] and therefore enhanced NO bioavailability. It was concluded that the biologically active components present in VCO, such as polyphenols or tocopherols (i.e. vitamin E) were likely the cause of this improvement as VCO possesses higher concentrations of these compounds than the other oils.[24]

Nair et al.[25] divided 24 male Balb mice with induced chronic inflammation into four groups: (I) control; (II) standard reference drug (i.e. 10 $mg \cdot kg^{-1}$ body weight diclofenac); (III) 4 g/kg body weight VCO; (IV) 8 $g \cdot kg^{-1}$ body weight VCO. After 20 days, the impact of each group on antioxidant status was assessed. It was found that liver and kidney catalase, superoxide dismutase and glutathione peroxidase activities were enhanced following 8 $g \cdot kg^{-1}$ body weight VCO, but not the other conditions.

Narayankutty et al.[18] provided 18 male Wistar rats with either a 10% VCO or CO diet for 4 weeks. The polyphenol content of the oils was assessed, and it was found that the content in VCO was almost double that of CO (32.2 ± 1.2 mg·100 g oil^{-1} vs. 18.1 ± 2.0 mg·100 g oil^{-1}). Similar to the previous two studies, it was found that the concentrations of superoxide dismutase, reduced glutathione, glutathione reductase, glutathione peroxidase, and catalase in the liver were all enhanced following VCO treatment.

Recently, several more animal studies have demonstrated that VCO can improve cognitive function,[21] reduce the incidence of oxidative stress-induced hepatic injury,[22] and mitigate stress by diminishing corticosterone and serum cholesterol levels.[23] The ability of VCO to upregulate antioxidants such as superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase was the proposed reason for these observed benefits.

Reports of improvements in antioxidant status via VCO in animal models are not uncommon. Several other studies have also investigated this phenomenon and have reported similar results.[17,27,106,124] However, the direct effect of VCO on vascular function and NO production is poorly understood, and has never been investigated in humans. If VCO polyphenols have the ability to promote antioxidant formation and the scavenging of ROS, it seems possible that it may elicit a significant positive effect on vascular endothelial function.

2.7.2 – VCO and Endothelial Function

A recent study conducted by Nurul-Iman et al.[28] was the first of its kind to investigate the effects of VCO on vascular reactivity and NO release (Figure 2.14). In this 16-week study, thirty-two male Sprague-Dawley rats were divided into four groups: control (basal diet), VCO (1.42 ml·kg^{-1} administered orally), 5HPO (five-times heated palm oil, 15%) and 5HPO + VCO.

Nitric oxide content was indirectly measured by determining plasma nitrite concentrations. Vascular reactivity was measured by exposing dissected thoracic aortic rings to the vasodilatory chemical acetylcholine, and the vasoconstricting chemical phenylephrine. The 5HPO group showed a significant decrease in NO content, attenuated vasodilation in response to acetylcholine, and enhanced vasoconstriction in response to phenylephrine. However, the 5HPO + VCO group and the VCO group showed a significant increase in NO content, and attenuated vasoconstriction in response to phenylephrine (Figure 2.14). This suggested that VCO possessed a strong ability to improve NO bioavailability and vascular reactivity, and it may counteract the negative effects of other pro-oxidative oils.

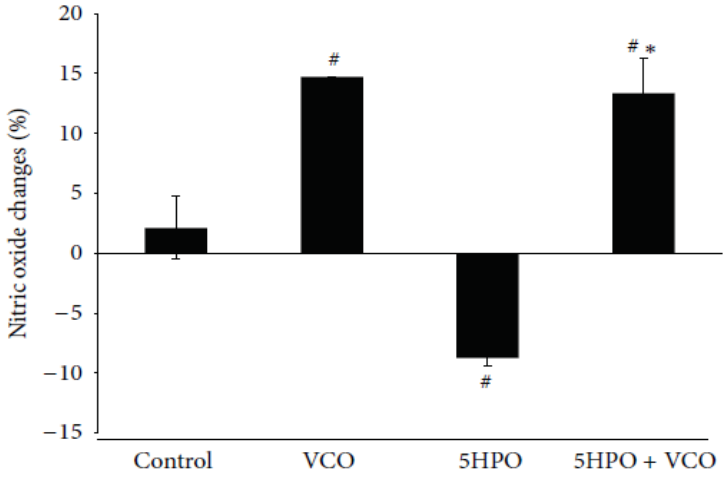

Figure 2.14: VCO increased the percent change in NO alone, and when combined with five-times heated palm oil. Five-times heated palm oil decreased nitric-oxide mediated changes when consumed alone. VCO, virgin coconut oil; 5HPO, five-times heated palm oil. #, $p < 0.05$ vs. control; *, $p < 0.05$ vs. 5HPO.[28]

A follow-up to the Nurul-Iman et al.[28] study was conducted by the same group in which thirty-two male Sprague-Dawley rats were once again divided into the same four groups for 16 weeks: control, VCO (1.42 ml·kg^{-1} administered orally), 5HPO (15%) and 5HPO + VCO.[32]

Kamisah et al.[32] assessed the protective effects of VCO on blood pressure and renal oxidation status. Measures of renal oxidation status included NO content indirectly measured by its metabolites plasma nitrite and nitrate, lipid peroxidation measured by thiobarbituric acid reactive substances (TBARS), and heme oxygenase activity (induced by oxidative stress) based on measurement of total bilirubin produced. The group fed 5HPO displayed elevated blood pressure, TBARS and renal NO content. However, when combined with VCO, the increase in blood pressure and renal NO content was attenuated. Both oils enhanced TBARS and reduced heme oxygenase when compared to the control group. Although it seems paradoxical that VCO decreased NO content while 5HPO increased it, the authors provided a reasonable explanation.[32] It was noted that the oxidative stress in the kidneys of the animals was likely elevated by the 5HPO, and this could downregulate eNOS and cause a responsive increase in inducible NO synthase (iNOS).[32,125] Enhanced iNOS activity can concurrently upregulate ROS during NO production and augment peroxynitrite formation, furthering oxidative stress.[126] Therefore, in this instance, enhanced renal NO production was not desirable, and VCO elicited a protective effect on eNOS. Overall, this study concurs with the previous [28] and demonstrates that VCO has strong antioxidant properties, even when combined with detrimental oils.

Other researchers have also investigated the effects of VCO on rats and concluded that the polyphenolic content of the oil may indirectly promote NO-induced vascular relaxation.[24,30] Although there have not been many studies published on the effects of VCO on vascular endothelial health, and none conducted in humans, there seems to be significant potential in this field.

If the dose (1.42 mL VCO·kg^{-1}) used in the previously presented studies [28,32] was applied to a 60 or 70 kg human, this would equal approximately 85.2 or 99.4 ml VCO per day,

respectively. Currently, the highest dose of VCO administered to humans in a peer-reviewed study has been 30 ml per day,[33,34,127] and this dose has been tested for its safety and efficacy in waist circumference reduction and lipid profile improvement.[33] Administering a dose approximately three times this amount was not possible before its potential toxicity in humans was established. Therefore, as significant improvements in waist circumference reduction and lipid profile have been demonstrated with a 30 ml·day^{-1} dosage of VCO in humans,[33,34,127] a dosage of 30 ml·day^{-1} was employed in the present study (see section 2.7).

2.7.3 – Lipid Oxidation

Virgin coconut oil can elicit significant inhibitory effects on lipid oxidation, while improving the overall lipid profile in animal models. Specifically, Narayankutty et al.[18] treated 18 male Wistar rats with either a 10% VCO or CO diet for 4 weeks and assessed the effect of these oils on lipid peroxidation, a hallmark for the preliminary stage of atherosclerosis. It was found that following the VCO diet, there was a significant reduction in lipid peroxidation (via reduced carbonyl formation).

Arunima & Rajamohan [105] conducted a study investigating the effects of a 45-day, 12g·day^{-1} diet with 8% VCO, CO or sunflower oil on lipogenesis and fatty acid catabolism in male Sprague-Dawley rats. It was found that compared to the other oils, VCO decreased tissue lipid levels and reduced the activity of enzymes involved in lipogenesis. Furthermore, VCO increased the mitochondrial and peroxisomal β-oxidation of fatty acids, indicating that the degradation of fatty acids was enhanced. Nevin & Rajamohan conducted three studies (2004, 2005, and 2008) in which male Sprague-Dawley rats were fed a 12g·day^{-1} diet with 8% VCO versus other oils such as CO and sunflower oil.[16,17,106] In their 2004 and 2008 studies,[16,106] it was found that VCO significantly reduced the magnitude of lipid peroxidation, as evidenced by

reduced carbonyl formation. The polyphenol fraction from VCO was found to have a significant advantage over other oils in preventing the oxidation of LDL because it traps the ROS in plasma and interstitial fluid of the arterial wall, thereby slowing their activity.[16] Additionally, when compared to CO, it was found that a diet supplemented with VCO significantly decreased total triglycerides, phospholipids, LDL-C and very low-density lipoprotein cholesterol (VLDL-C) levels.[16] These findings were supported by their second study in 2005 in which it was also found that VCO lowered the levels of total cholesterol, LDL-C and VLDL-C more than CO and sunflower oil.[17] Virgin coconut oil was able to increase high-density lipoprotein cholesterol (HDL-C) while concurrently decreasing LDL-C, creating a much healthier lipid profile in the Sprague-Dawley rats.[16] This ability to increase HDL-C is significant because higher levels of HDL-C support an increase in endothelial function, while lower levels of HDL-C can impair NO bioavailability.[128,129] The mechanism by which HDL-C improves endothelial function is unclear, however it may be due to its role in reverse cholesterol transport, or its action as an antioxidant.[129]

Brachial artery FMD and LDL diene conjugation (i.e. marker of *in vivo* LDL oxidation) were assessed in two groups of healthy men (< 40 years) with predetermined values [130] of HDL-C.[129] One group had high HDL-C and one group had low HDL-C. The group with high HDL-C had normal FMD values and significantly lower levels of LDL oxidation. However, the group with low HDL-C had impaired FMD and significantly higher levels of LDL oxidation compared to the high HDL-C group.[129] This indicates that HDL-C may provide protection to the endothelium and inhibit LDL oxidation.

Similarly, Lupattelli et al.[128] examined FMD of otherwise healthy hyperlipemic patients (30-68 years) with low or high HDL-C levels. It was found that low HDL-C concentrations were

associated with impaired brachial artery FMD, and that as HDL-C concentrations increased, so did FMD (Figure 2.15).

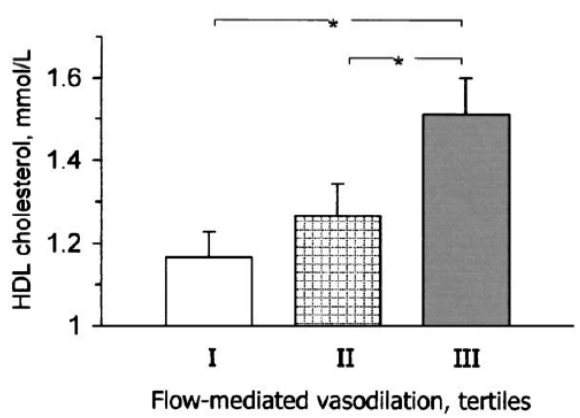

Figure 2.15: HDL cholesterol values by tertiles of brachial artery FMD. I = < 2.8%, II = 2.8 - 4.8%, III = > 4.8%. HDL, high-density lipoprotein. *, $p < 0.05$, HDL cholesterol levels in III were significantly greater than both I and II.[128]

2.7.4 – VCO and Exercise

A novel aspect of the present study will be to observe the effects of VCO on arterial blood flow following a single bout of moderate-intensity aerobic exercise. A study on hypertensive rats was conducted by Alves et al.[131], examining the effects of 4-week, 2 ml·day^{-1} VCO alone, or in combination with swim training on mean arterial pressure measured through a catheter in the femoral artery, and oxidative stress measured by malondialdehyde (a marker of lipid oxidation) and superoxide accumulation. The rats were divided into 5 groups: Wistar Kyoto rats + saline (control), hypertensive rats + saline, hypertensive rats and VCO, trained hypertensive rats, and trained hypertensive rats + VCO. Mean arterial pressure decreased the furthest in the trained hypertensive rats + VCO group. Virgin coconut oil alone was most effective at decreasing malondialdehyde (a marker of lipid oxidation) followed closely by the trained + VCO and trained groups (Figure 2.16A). Additionally, VCO alone, trained + VCO and

trained groups were also equally effective at significantly preventing superoxide accumulation when compared to the hypertensive + saline group (Figure 2.16B).

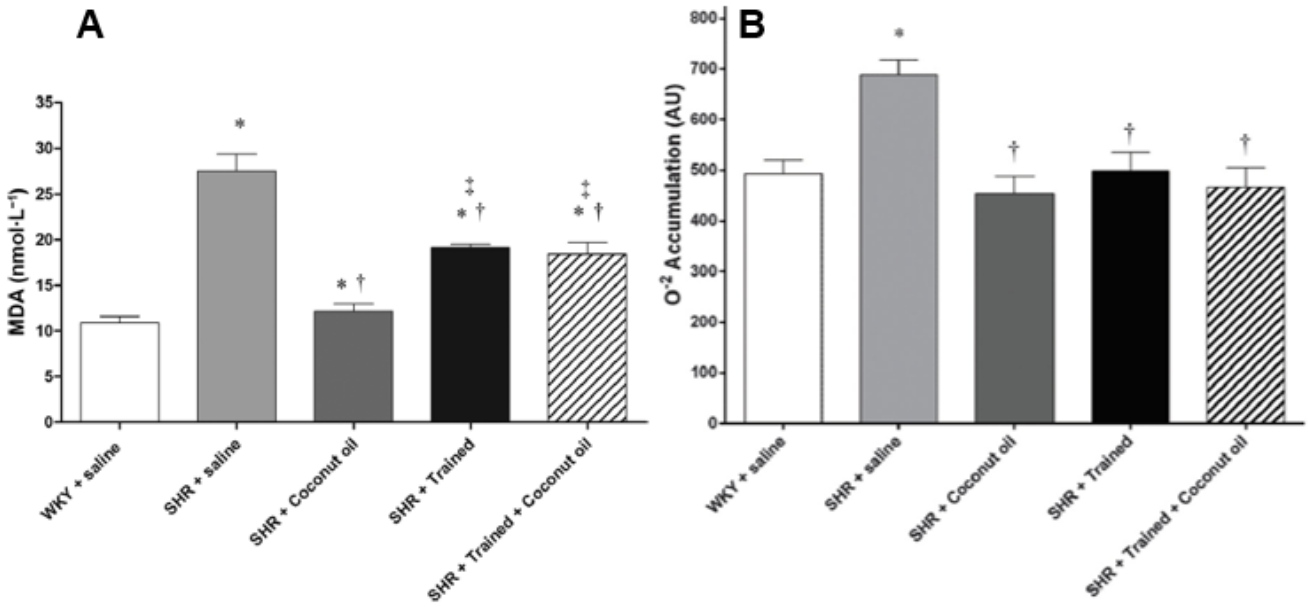

Figure 2.16: VCO (labelled as coconut oil) supplementation alone was the most effective at reducing malondialdehyde (A) and superoxide (B) concentrations in hypertensive rats, following by training + VCO and training alone. MDA, malondialdehyde; WKY, Wistar Kyoto rats; SHR, spontaneously hypertensive rats; VCO, virgin coconut oil. *, $p < 0.05$ vs. WKY + saline; †, $p < 0.05$ vs. SHR + saline; ‡, $p < 0.05$ vs. SHR + coconut oil.[131]

This study differs from the present as the use of exercise training was employed in rat models, rather than a single bout of moderate intensity exercise in humans. Furthermore, post exercise-mediated hyperemia was not examined in this study. Examining the effect of VCO on exercise-mediated hyperemia in a human model will be a novel and clinically relevant aspect of this research project.

2.8 – VCO Human Studies

As eluded to previously, the available literature regarding the use of VCO on health parameters in humans is sparse. Law et al.[35] tested the effect of VCO on the quality of life in breast cancer patients. The participants ingested 10 ml of VCO twice daily as a supplement for

one week following six rounds of chemotherapy. It was found that VCO consumption enhanced overall quality of life by improving common side effects of chemotherapy such as fatigue, dyspnea, sleep and loss of appetite.

Liau et al.[33] administered 30 ml VCO (divided in 3 doses, consumed a half an hour before each meal) for 4 weeks to 20 healthy adults (20-60 years). It was found that compared to a control group (no VCO), the waist circumference of these individuals decreased significantly following VCO ingestion. This potentially indicates that VCO elicited a significant positive effect on fatty acid catabolism comparable to what was previously observed in animal models.[17,24,106] However, a very recent crossover study by Harris et al.[34] also administered 30 ml VCO or safflower oil for 4 weeks to 12 postmenopausal women (59 ± 4 years) and assessed their body composition, lipids and inflammatory markers. VCO was shown to decrease inflammatory biomarkers such as interleukin-1β in some participants, and increase total cholesterol, LDL-C and HDL-C, while maintaining body composition, of all participants. No significant changes in these parameters were observed with safflower oil ingestion. Another very recent crossover study compared the effects of an acute dose of 25 ml VCO or olive oil on resting energy expenditure, fat oxidation rate, diet induced thermogenesis, appetite and lipid parameters in adult women with excess body fat (37.43 ± 0.83%).[132] No differences were reported between the two oils in any measures, and VCO promoted less appetitive responses. However, the choices to only administer an acute dose of VCO and use olive oil as a control are questionable, as olive oil ingestion has been correlated with cardiovascular disease risk reduction,[133] and no other study has investigated the effect of an acute dose of VCO. Therefore, there is still significant evidence that VCO elicits beneficial effects on cardiovascular function.

In support of this, Cardoso et al.[134] assessed waist circumference in 116 patients with coronary artery disease (62 ± 8 years, 63% male) following 13 ml·day^{-1} (divided in 3 doses, ingested a half an hour before each meal) of VCO for three months. It was also concluded that there was a significant decrease in waist circumference in these individuals, and additionally, it was found that there was a significant increase in HDL-C, similar to Harris et al.,[34] and Nevin & Rajamohan.[17] Therefore, VCO may exert a beneficial effect on the lipid profile in humans and may subsequently improve endothelial function in a population with CVD. Although these individuals had CVD and the duration of ingestion was long, improvements in the lipid profile of healthy adults following shorter durations of VCO treatment have been reported. Specifically, Assuncao et al.[127] administered 30 ml·day^{-1} (divided in 3 doses, consumed with each meal) of coconut oil or soy bean oil for 12 weeks in 40 healthy women (20-40 years) and assessed the impact on their biochemical (i.e. LDL to HDL ratio) and anthropometric (i.e. waist circumference) profiles. Additionally, the women were asked to walk 50 min per day. Similar to Cardoso et al.,[134] a significant reduction in waist circumference accompanied by a significant rise in HDL-C and an improved overall lipid profile following coconut oil ingestion was reported. Importantly, this study employed the use of coconut oil rather than VCO. As such, there is potential that greater beneficial effects on lipid profiles may have been observed had VCO been used instead.

A final study also investigating the use of coconut oil in human health was by Voon et al.[135] who incorporated coconut oil, virgin olive oil, and palm oil into the diets of 45 healthy Malaysian adults (20-60 years, 36 female). Coconut oil, virgin olive oil or palm oil were ingested as 2/3 of the 30% fat portion of the diet for five weeks. It was found that following the coconut oil diet, but not the virgin olive oil or palm oil diets, there was less platelet adhesion observed in

the cells, indicating a reduced risk of atherosclerosis in these individuals. These results collectively suggest that VCO may elicit significant positive benefits on health parameters related to endothelial function, and therefore NO production.

However, despite these conclusions, the American Heart Association (AHA) recently issued a statement indicating that foods with a high saturated fat content (i.e. coconut oil, beef fat and butter) should be classified as unhealthy foods that increase the risk of CVD.[136] This is unfortunate because the AHA failed to take into consideration the nutritional density present in coconut oil when compared to the other highly saturated fats.[7,8] Additionally, the AHA did not consider VCO specifically. VCO has a higher polyphenol content than coconut oil,[8] and it possesses antioxidant,[16,17,20,24] anti-inflammatory,[30] and anti-bacterial properties.[137] Instead of deeming the entire oil unhealthy, perhaps future recommendations should include extracting the polyphenol fraction of VCO to be used as a natural health product.

2.9 – Dosage and Duration

As previously described, a 30 ml (~2 tablespoons) daily dose of VCO elicits positive health effects in healthy humans.[33,34,127] Additionally, the majority of research on the effects of VCO ingestion on endothelial function and resistance of lipids to oxidation demonstrate enhanced effects with short (< 1 month) [33,34,131] or long-term (> 1 month) [28] administration. Based on previous rodent [18,131] and human [33,34] studies that have observed changes in physiologic function following four weeks of VCO ingestion, the effects of a four-week VCO ingestion period on blood vessel function was investigated. This time period was also feasible for a Master's level thesis project.

2.10 – Measures of Vascular Endothelial Function

Endothelial dysfunction is the characteristic first stage of CVD.[3] Early indications of CVD can occur long before the appearance of plaque accumulation or chest pain,[138,139] therefore tests that can detect these early signs can be of great benefit.

In order to observe and measure endothelial function of an artery, high-resolution duplex ultrasound imaging was required. Duplex ultrasound is recommended because it allows simultaneous observation of images of the artery in brightness mode (B-mode) and blood flow velocities using pulsed-wave Doppler [140] (Figure 2.17).

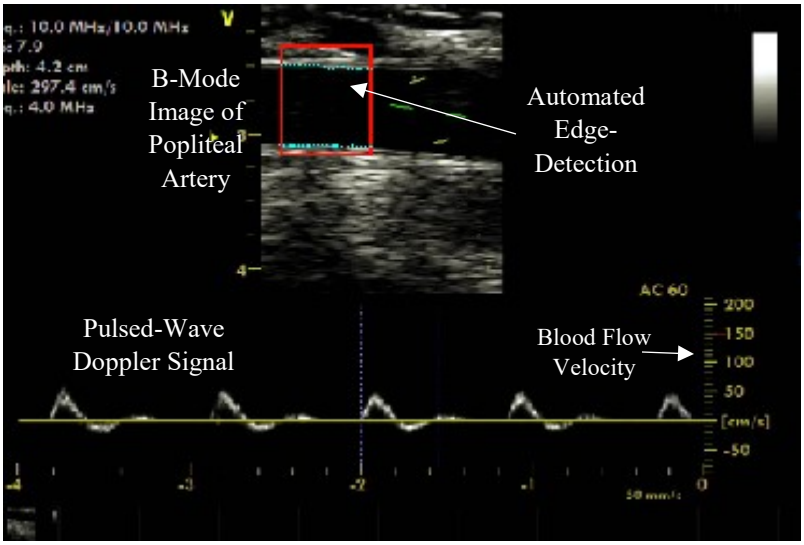

Figure 2.17: Example of the information observed from high-resolution duplex ultrasound imaging using both B-mode imaging and pulse-wave Doppler signals. B-mode, brightness mode.

B-mode imaging creates a high-resolution two-dimensional image that allows the technician to view the intima-lumen interface at the anterior and posterior walls of the artery. These interfaces are termed the double lines of Pignoli [141] (Figure 2.18), and are where the measures of arterial lumen diameter are obtained. In addition to the high-resolution ultrasound

machine, automated edge-detection software is used (Figure 2.17), which accurately and reliably determines the changes in arterial lumen diameter.[142–144]

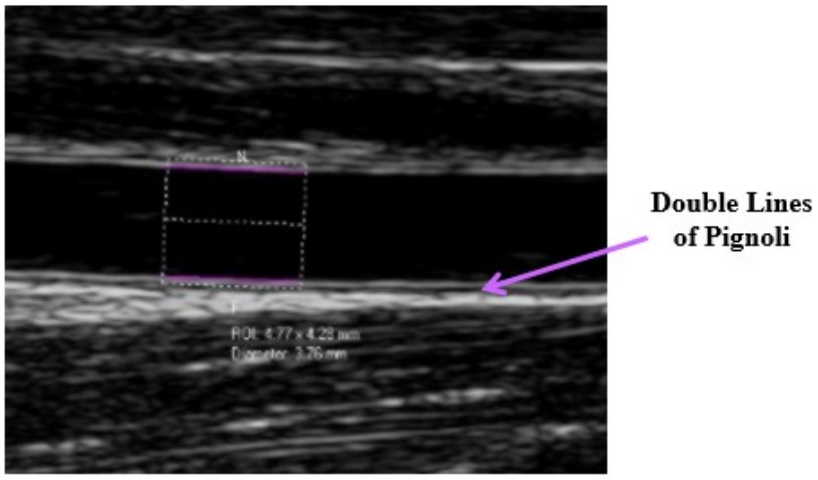

Figure 2.18: B-mode image of an artery displaying the double lines of Pignoli and use of automated edge-detection software. B-mode, brightness mode.

Pulsed-wave Doppler velocity signals were used to determine the velocity of blood flowing through the artery. The linear array transducer (i.e. the hand-held probe) contains two piezoelectric crystals which transmit and receive signals. Pulsed-wave Doppler sends a pulsed signal to a certain depth and allows the reflected wave to return to the transducer before the second pulse is generated (Figure 2.19). In the present study, this pulsed wave technique allowed the calculation of blood flow velocity.[140]

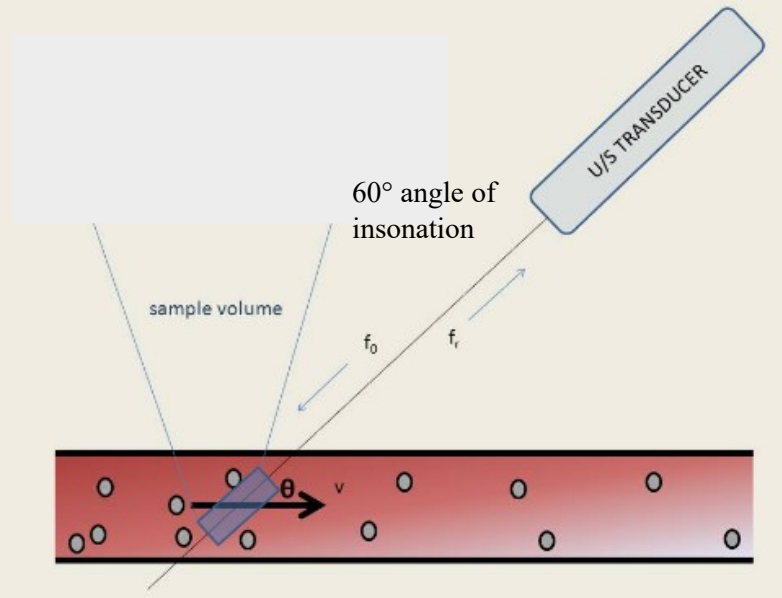

Figure 2.19: Example of pulsed-wave Doppler transmitting a signal and receiving it back to the transducer at a 60° angle of insonation. U/S, ultrasound; f_0, transmitted frequency; f_r, returning frequency; θ, angle of insonation; v, velocity.[145]

In diagnostic ultrasound, a small sample size, or sampling volume, is placed directly in the center of the vessel to determine if a stenosis (i.e. blockage) exists [140] (Figure 2.20A). Blood flows through the vessels in a laminar fashion (i.e. fastest velocities in the center) and fast velocities may indicate compensation by the system to account for blockage. However, when obtaining the average blood flow velocity of the vessel, it is better to make the sample size larger, and have it stretch from the anterior wall to the posterior wall [140] (Figure 2.20B). Therefore, in the present study, a sample size that encompassed the anterior to posterior intimal layers was used to record red blood cell velocity.

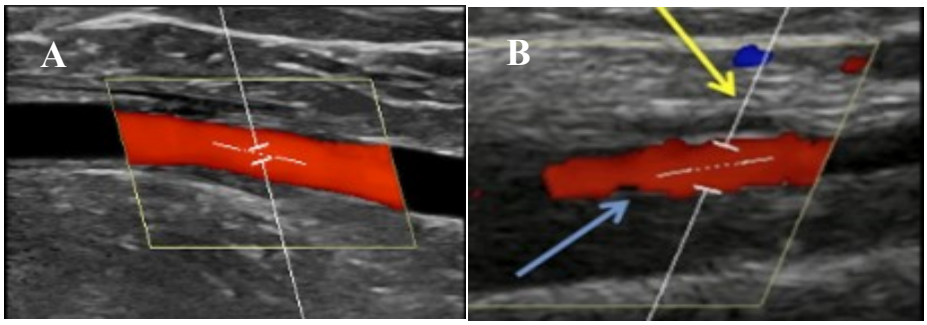

Figure 2.20: Images of a sample volume on an artery. A) small sample size used for diagnosis of stenotic lesions detected by fast velocities; B) large sample size to obtain the average blood flow.

2.10.1 – Flow-Mediated Dilation

The FMD technique is a surrogate measure of NO production, and allows for the early detection of endothelial dysfunction.[2] Celermajer et al.[138] was the first group to devise the non-invasive FMD technique. The technique was used to assess whether abnormalities in endothelial function were present in children and young adults who were at risk of developing CVD, but who were free of symptoms. It was found that although these individuals did not display outward symptoms of CVD, such as plaque in their blood vessels, their endothelial function was impaired. Thus, FMD became a viable non-invasive measure of CV function. Since this study was published many other researchers have used FMD in an attempt to predict CVD.[144,146] Inaba et al.[146] composed a meta-analysis of observational studies examining the relationship between FMD of the brachial artery, and future CV events. Fourteen cohort studies with 5,547 participants were included in the analyses. Significant associations between brachial artery FMD and CV events were found, and it was concluded that impairment of brachial FMD is a strong predictor of these events. Flow-mediated dilation has also been deemed an important predictor of coronary risk factors in asymptomatic patients,[147] and a predictor of cardiovascular events in patients with peripheral artery disease,[148] and coronary artery disease.[149]

The main purpose of the FMD test is to quantify the ability of the artery in question to produce NO and cause VSM relaxation.[36] The first step of the FMD test involves the inflation of a pressure cuff, to a supra-systolic pressure, distal to the artery for five minutes. The pressure cuff is inflated distally to the artery in order to ensure that the FMD response is primarily caused by the action of NO. Doshi et al.[150] compared a distal cuff placement to a proximal cuff placement and observed detectable differences. It was found that there was a larger FMD response with a proximal cuff placement than a distal placement. However, it was later determined that the proximal occlusion was not solely NO-mediated.[150] When an artery is occluded proximally, metabolites that trigger eNOS activity (i.e. histamine and bradykinin) or vasodilators (i.e. prostaglandins and adenosine), can be produced by the artery to maintain vasodilation and enhance blood flow [150–152] (see sections 2.1.3 and section 2.3). Therefore, when the cuff is released, not only is shear stress-triggered NO production causing vasodilation, the other vasodilators are contributing as well. Distal cuff inflation isolates endothelial-mediated dilation, as the shear stress associated with the post-cuff deflation increase in blood flow (i.e. hyperemia) is the predominant stimulus for NO production and vasodilation. Local metabolite accumulation (e.g. histamine and bradykinin) occurs downstream from the measurement site and does not contribute. Doshi et al.[150] used L-NMMA, in both distal and proximal cuff occlusions to assess this hypothesis. It was found that the FMD response was abolished in the distal cuff occlusion condition following L-NMMA administration. However, L-NMMA infusion did not affect the FMD response using the proximal cuff occlusion protocol. This indicated that vasodilation following a distal occlusion is almost solely mediated by NO.

An infusion of L-NMMA has also been used to support the use of the five-minute period of cuff occlusion. Peer-reviewed publications have demonstrated that an infusion of L-NMMA

prior to an arterial occlusion period of 5 minutes abolished the FMD response of the radial artery.[153,154] However, when the FMD test was performed using a 15-minute cuff occlusion period, the FMD response of the radial artery was unaffected indicating an NO-independent mechanism was involved with the hyperemia-induced vasodilation.[153,154] Mullen et al.[154] concluded that during sustained periods of reduced NO synthesis, it is possible that alternate mechanisms, such as local ischemia, vasoactive metabolites, or neuronal mechanisms are compensating and may reduce the immediate effectiveness of NO.[154] Therefore, based on these results, it is recommended to employ a shorter occlusion period of five minutes.

Following cuff deflation, artery diameter and blood flow velocity are measured for 3-5 minutes. Thijssen et al.[144] composed a review of various technical approaches to FMD measurement and analysis, and compiled recommendations for lower body artery FMD. Population characteristics were not provided in this review. However, the same group investigated PA (i.e. artery assessed in current study) vasodilatory capacity in healthy young individuals (23-36 years) using these technical approaches, and obtained relative FMD (i.e. FMD%) values ranging from 6.1 ± 3.3% [155] to 6.7 ± 3.3% [156]. Larger arteries in the lower limbs reach peak dilation later than smaller arteries in the upper limbs.[155] To assess endothelial-dependent dilation and observe a true peak in a lower limb artery, it is recommended that arterial diameter be measured for a minimum of 5 minutes, rather than the typical 3-minute recommendation for upper limb arteries.[144] Larger increases in arterial diameter following cuff deflation indicate an increased ability of the endothelium to produce NO and/or enhanced sensitivity of the VSM to relax in response to an increased availability of NO.

Studies have shown that differences exist in the FMD response between various arteries.[144,155,157,158] The percent change in FMD (FMD%) between various arteries was evaluated

in healthy, recreationally active non-smoking men (31 ± 7 years).[155] In small upper limb arteries, FMD% was found to be greater (7.0 ± 2.4% brachial artery; 9.4 ± 3.0% radial artery) than the larger lower limb arteries (3.9 ± 1.9% femoral artery; 6.9 ± 2.7% superficial femoral artery; 6.1 ± 3.3% popliteal artery).[155] Normalizing the magnitude of the relative FMD response (i.e. the percent increase from the baseline diameter) to the shear rate area under the curve (SR_{AUC}) from the time of cuff deflation to the time that maximal vasodilation occurs can account for these differences [71,159,160] (Figure 2.21).

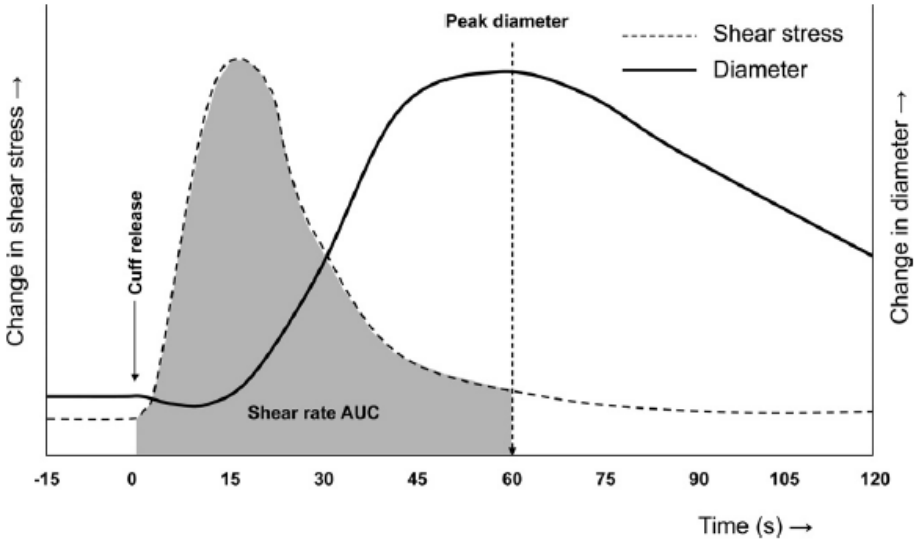

Figure 2.21: Normalizing FMD involves dividing the relative FMD response (i.e. the percent increase from the baseline diameter) by the shear rate area under the curve. AUC, area under the curve.[144]

2.11 – Research Questions

The aim of the present study was to determine whether a four-week ingestion of VCO (30 $ml \cdot day^{-1}$) in young, healthy adults could enhance resting PA FMD and augment exercise-mediated hyperemia following a single, brief bout of moderate-intensity cycling exercise. Additionally, it was investigated whether endothelial-independent dilation (NTG) and plasma

TAC were significantly elevated following this period of VCO ingestion. If all outcomes were enhanced, this would provide support for the use of VCO in the prevention of endothelial dysfunction.

Chapter 3: Method of Procedure

3.1 – Participants

To estimate sample size, a previous human investigation was considered [33] that documented the efficacy and safety of a four-week (30 ml·day^{-1}) ingestion of VCO on reducing visceral adiposity (i.e., waist-to-hip ratio) in a population of middle-aged (40 ± 9 years, 24-51 years) obese but otherwise healthy adults. Increased abdominal adiposity determined by the waist-to-hip ratio is a strong predictor of vascular endothelial dysfunction in healthy overweight adults.[161] In addition, a four-week VCO ingestion study conducted in rodents was considered [18] that documented a significant increase in antioxidant enzyme activity (e.g., superoxide dismutase, SOD). Specifically, compared to the control group (79.28 ± 4.29 U·mg^{-1} protein), SOD activity increased to 87.51 ± 5.74 U·mg^{-1} protein in the group that ingested VCO for four weeks. These means and standard deviations, were inputted into a sample size calculation (http://www.uccs.edu/~lbecker/), which produced an effect size value of 0.81. This value was inputted into a power calculator (G Power v3.1.3) using a dependent t-test design with an alpha error probability of 0.05 (i.e., Power = 0.95). This *a priori* analysis resulted in an estimated sample size of 20. Overall, 24 healthy young adults (18-30 years) were recruited for this study. Five participants dropped out due to various reasons, therefore 19 participants were included in the analysis.

To be eligible for the study, participants had to be healthy young adults between the ages of 18 and 30 years with a body mass index (BMI) less than 30 kg·m^{-2}. Participants could not be hypertensive, taking blood pressure medication, or smokers. Additionally, participants had to be

free of chronic disease, and able to exercise comfortably. Female participants could not be pregnant or breastfeeding, and had to have a regular 28-day menstrual cycle.

Since this study was the first to assess the impact of VCO on endothelial function and exercise-mediated hyperemia in humans, a population of young, healthy individuals was investigated. Oxidative stress can occur in all individuals for various reasons (i.e. aerobic metabolism, air quality, smoke, poor diet, etc.).[48] Therefore, it was conceivable that VCO would elicit a positive effect on arterial function in young, healthy individuals. Once more is known regarding the effect of VCO in a young, healthy population, future recommendations for testing the effect of VCO in populations with elevated ROS levels (i.e. elderly or diseased) can be made.

3.2 – Recruitment

Participants were recruited through posters emailed to Kinesiology students and attached to notice boards throughout Dalhousie University including the Dalplex, Life Sciences Centre, and Student Union Building (Appendix A). These posters included e-mail information of the principal investigator, Susan Robinson. It was made clear to all students that their decision to participate, or not, would result in no beneficial or adverse academic outcomes.

The principal investigator conducted all recruitment and provided all participants with the Information Letter and Informed Consent document (Appendix B), a Health History Questionnaire (Appendix C), and the Physical Activity Readiness-Questionnaire (PAR-Q, Appendix D). The Health History Questionnaire (Appendix C) contained information to calculate BMI, and a checklist of exclusion criteria. A checklist of exclusion criteria was also provided on the consent form (Appendix B).

3.3 – Experimental Design

The study was a single-group pre-test, post-test experimental design (Figure 3.1). There were no blinding procedures involved with this protocol. The entire study required three separate visits to the laboratory and a total time commitment of ~4 hours. Days 1 and 2 were separated by a minimum of two weeks, and Days 2 and 3 were separated by minimum of four weeks. To avoid confounding influences on cardiovascular function associated with diurnal variation, all experimental sessions were held at approximately the same time of day. Twenty-four hours prior to each visit, participants abstained from alcohol, and caffeine consumption, as well as the engagement in vigorous physical activity (i.e., activity in which participants cannot engage in a conversation while performing). Participants arrived at each session well rested (~8 hours of sleep), well hydrated, and ~3 hours after consumption of their last meal. Female participants underwent their pre- and post-VCO sessions during the same phase of their menstrual cycle. Specifically, to avoid any potential confounding influences of the menstrual cycle on blood vessel function,[162] women were assessed on days 1-7 of the menstrual cycle or during the placebo phase of oral contraceptive use

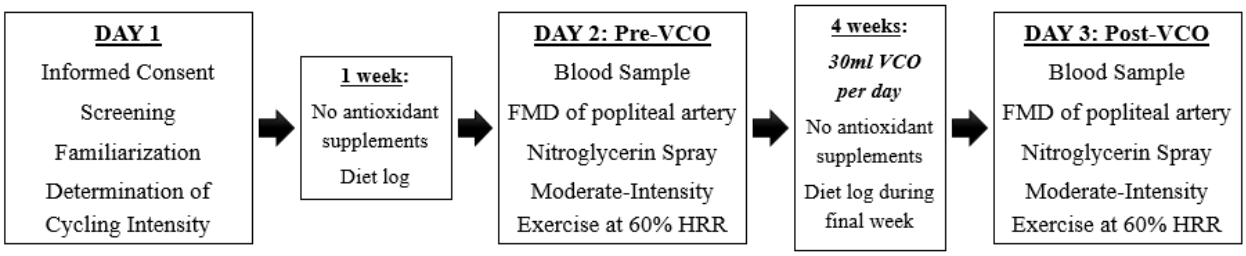

Figure 3.1: Overview of pre-VCO, post-VCO experimental design. VCO, virgin coconut oil; FMD, flow-mediated dilation; HRR, heart rate reserve.

Flow-mediated dilation results can be influenced by certain foods. As such, all participants were asked to avoid the consumption of chocolate and kiwi for 8 hours before their

second and third visits.[163,164] Additionally, all participants were asked to avoid the consumption of antioxidant supplements, such as vitamin E or C, CoQ-10 (ubiquinone), alpha-lipoic acid, grape seed extract (resveratrol), carotenoids, green tea extract, L-glutathione, and quercetin for the duration of the study following Day 1 as these can interfere with, or mask the effect of, the antioxidants present in VCO. Participants were also asked to record their daily food intake for one week prior to Days 2 and 3 (Appendix E). Their intake of starches, meat & alternatives, fruits, vegetables, milk & alternatives, fats, sugary foods and alcohol for each week were calculated by the principal researcher based on serving sizes found in the official Diabète Québec Meal Planning handbook. These values were documented and average daily intakes were calculated. Participants were provided with their average daily and weekly intake prior to Day 2 and asked to maintain a similar diet composition before Day 3. Both diet logs were compared. These instructions helped minimize confounding factors that could have influenced blood vessel function and exercise-mediated blood flow responses. Additionally, maintaining a consistent diet composition helped avoid excess antioxidant ingestion by the participants and ensured that any increases in total antioxidant content are mediated primarily by VCO. Finally, participants were asked to maintain their normal exercise regime (i.e. through self-reporting) for the duration of the study in order to minimize any effect of training on endothelial function.

3.4 – Experimental Protocol

3.4.1 – Day 1: Familiarization and Determination of Cycling Intensity

Following informed consent and familiarization with all testing equipment and procedures, participants were instrumented with a wireless chest strap heart rate (HR) monitor (T31 coded^{TM} transmitter, Polar®). The participants sat on an electronically-braked cycle ergometer (Excalibur, Lode) and had the seat height adjusted to ensure a slight knee bend (~10-

20° knee flexion) when the pedals were at the lowest position. This seat height was recorded for use on Days 2 and 3 to ensure consistency across all three cycling sessions. Participants remained seated for two minutes to allow their HR to stabilize and attain resting HR values, which were used to help determine their target exercise HR (see below). Target exercise HR was calculated using the Karvonen or heart rate reserve (HRR) method.[165] Heart rate reserve was calculated as the difference between their age-predicted maximum HR and their resting HR. Specifically, subjects exercised at an intensity that elicited 60% HRR using the following equations:

$$\text{Target HR} = [0.6 \times (\text{Age-predicted maximum HR} - \text{Resting HR})] + \text{Resting HR},$$

$$\text{where Age-predicted maximum HR} = 207 - (0.67 \times \text{Age})$$ [166]

The workload on the cycle ergometer was first set to a low level (~20-50 Watts) for a five-minute warm-up. Every three minutes, the workload was increased by 10-30 Watts until the participants were within ± 5 beats·min^{-1} of their target HR over the final minute of the last three-minute stage. The workload achieved to reach their target HR was recorded. Participants then completed a minimum five-minute recovery period at the same intensity used during the warm-up. Following a resting period of five minutes, participants performed a confirmation cycling test in which they exercised at the target workload for at least five minutes to ensure that their steady-state target HR was achieved. If target HR was outside the ± 5 beats·min^{-1} acceptable range, the workload was adjusted accordingly. The final workload used to achieve the target HR was recorded and used during the exercise bouts on Days 2 and 3.

3.4.2 – Days 2 and 3: Pre-VCO and Post-VCO

The first part of Days 2 and 3 involved the collection of a single blood sample (0.4 ml) from each participant via finger prick while they were in a right-lateral recumbent position (see Section 3.5.1). Following the blood collection, resting HR and blood pressure were measured. Both HR and BP measures were taken while participants were in the prone position. Participants instrumented themselves with a wireless chest strap HR monitor (T31 coded™ transmitter, Polar®) around the level of the xiphoid process. Resting arterial blood pressure was measured in the left brachial artery (Carescape™ v100, General Electric Healthcare) following a 5-minute minimum resting period, and a Portapres® (Finapres Medical Systems) non-invasive blood pressure monitor provided continuous measurements of arterial pressure from the right index or middle finger throughout each test. The Portapres® device was removed while participants exercised. The Carescape™ blood pressure recording was used to re-calibrate the Portapres® waveform for the most accurate blood pressure readings. Briefly, a representative single beat of the Portapres® recording occurring immediately prior to the Carescape™ blood pressure recording was selected. The maximum (systolic) and minimum (diastolic) points of this beat were then calibrated to the corresponding Carescape™ values. A detailed schematic of the experimental protocol is presented in Figure 3.2.

3.4.2.1 – Popliteal Artery Endothelial-Dependent Dilation

For the PA-FMD procedure, participants were in the prone position and had foam pads placed above and below their left knee to ensure that their leg remained stationary and comfortable for the full FMD test (~12 minutes). A pressure cuff was positioned below the PA around the mid-calf and connected in series to a rapid cuff inflation system (E20 and AG101, Hokanson®, Bellevue, WA).

Although the brachial artery has been extensively researched, the PA was chosen because lower extremity branches are more susceptible to atherosclerotic lesion formation than those of the upper extremity.[167,168] It was useful to measure endothelial function of a more susceptible artery, as VCO would likely exert a more powerful effect on this artery. Additionally, the participants would be primarily using their lower body during exercise (i.e. cycling). Therefore, it was prudent to assess a lower body artery.

Blood flow and PA diameter were recorded using a 12 MHz multi-frequency linear array transducer probe connected to a high-resolution ultrasound machine (Vivid i, General Electric Healthcare). Water-based conductive gel was applied to the 12 MHz ultrasound probe to transmit the high-frequency sound waves safely and effectively through the skin when the probe was pressed against the skin surface. The probe position was carefully adjusted until the optimal longitudinal view of the PA was found, while maintaining an angle of insonation $\leq 60°$ to collect the best possible B-mode image and Pulsed-Doppler waveform. Once an acceptable PA image was attained, the probe location was traced on the skin for quicker image acquisition times during the subsequent tests.

Two minutes of resting PA diameter and red blood cell velocity were recorded before the pressure cuff was rapidly inflated to 250 mmHg for five minutes. This produced a temporary period of ischemia (i.e. blood flow restriction) and resulted in vasodilation of the arterioles in the lower calf and foot which lead to a subsequent increase in PA blood flow following cuff deflation. The resulting increase in PA blood flow (and shear stress) is the stimulus responsible for the production and release of NO from the vascular endothelial cells, which produced the corresponding vasodilation. Continuous measures of PA diameter and red blood cell velocity

were recorded both during the cuff inflation period and for an additional five minutes after the cuff was released. This ensured the capture of peak PA diameter.[144]

3.4.2.2 – Popliteal Artery Endothelial-Independent Dilation

Previous research has shown that at least 10 minutes of rest is needed following cuff deflation to allow the artery diameter and blood flow to return to baseline levels.[169] Therefore, participants were provided with a minimum 10-minute recovery period following the FMD assessment. Endothelial-independent vasodilation of the PA was then examined following a 0.4 mg dose of NTG sprayed under their tongue. NTG acts as a 'donor' of NO and is provided to determine the maximal vasodilatory response of the PA, and importantly, to assess the sensitivity of the VSM to respond (i.e. relax) to NO. Prior to the administration of NTG, a one-minute baseline assessment of PA diameter and red blood cell velocity was collected using the ultrasound machine. These measurements were also continuously recorded for 10 minutes after the NTG spray was administered.

3.4.2.3 – Popliteal Artery Post-Exercise Hyperemia

Following the NTG test, participants sat on the cycle ergometer with the same seat height determined on Day 1. The workload was set at ~30-50 watts and the subjects warmed-up at this intensity for 5 minutes. At the end of the warm-up period, the workload on the ergometer was immediately increased to the level determined on Day 1 to achieve their target HR (i.e. 60% HRR). Participants continued to cycle at this intensity for 10 minutes. Small adjustments were made to the workload if exercising HR was greater or less than 5% of the target value.

The exercise duration and intensity were chosen to comply with the minimum recommendations (i.e. 10-minute bout of moderate-to-vigorous intensity exercise) of the

Canadian Society for Exercise Physiology (CSEP) and the American College of Sports Medicine (ACSM). Additionally, an intensity of 60% elicits a hyperemic response, and a 10-minute duration is long enough to achieve a steady-state HR. Finally, the HRR method was chosen to account for individual differences in participant resting HR.[165]

Immediately after the 10 minutes of cycling was complete, their PA diameter and red blood cell velocity were recorded for five minutes while they remained seated on the cycle ergometer with their left leg extended and the pedal at the lowest position to ensure the most accurate assessment of peak post-exercise blood flow. Bernick et al.[170] assessed popliteal artery blood flow immediately following 3-minutes of exercise on a calf ergometer (remained on ergometer) using Doppler ultrasound. It was found that post-exercise hyperemia was highest immediately following exercise.[170] Moving participants from the cycle ergometer to the testing room could elicit a value less than the true peak. Additionally, blood flow velocity and PA diameter were recorded for five minutes following exercise. This allowed sufficient time for endothelial function and CV parameters to return to resting levels.[51] Once their HR and blood pressure returned to resting levels they were permitted to leave the laboratory.

To determine the magnitude that exercise-hyperemia was increased following VCO supplementation, resting PA blood flow velocity and blood flow were recorded. Villar & Hughson [171], assessed PA blood flow velocity and blood flow using a Doppler ultrasound machine in a resting baseline condition in healthy (28 ± 3 years) young adults. Popliteal blood flow velocity ($cm \cdot sec^{-1}$) ranged from 2.7 ± 0.8 to 2.8 ± 1.0, and popliteal blood flow ($ml \cdot min^{-1}$) ranged from 52.1 ± 15.4 to 52.5 ± 20.6.[171] Additionally, a change in PA exercise-mediated blood flow of approximately 80-100 $ml \cdot min^{-1}$ [171,172] was used to ensure a reasonable hyperemic response was obtained.

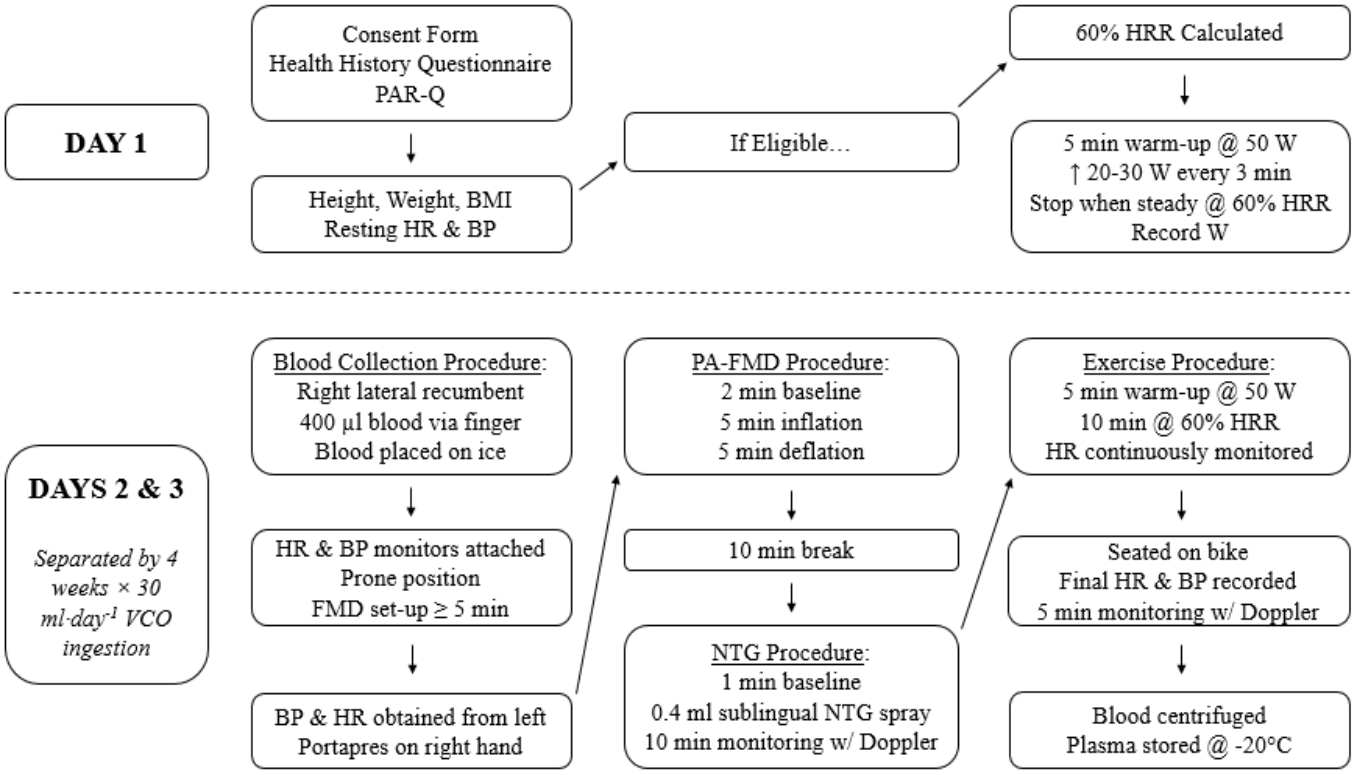

Figure 3.2: Detailed experimental protocol. PAR-Q, physical activity readiness questionnaire; BMI, body mass index; HR, heart rate; BP, blood pressure; HRR, heart rate reserve; W, watts; VCO, virgin coconut oil; FMD, flow-mediated dilation; PA-FMD, popliteal artery flow-mediated dilation; NTG, nitroglycerin.

3.4.2.4 – Dietary Supplementation and Instructions

Before leaving the laboratory on Day 2, participants were provided with their four-week dose (30 ml or 2 tablespoons·day^{-1}) of VCO (Progressive® Organics Organic Virgin Coconut Oil, 908 grams) and scheduled for their Day 3 visit. The final dose of VCO was to be consumed the day before the participants arrived at the lab for their Day 3 visit. Participants were instructed that they may either consume the VCO alone or with food such as adding to a smoothie or a pre-cooked stir-fry, and they could consume the VCO at any time during the day. Participants were not allowed to cook with the VCO as burning or repeatedly heating the VCO may potentially decrease the antioxidant content of the oil.[173] Examples of recipes (Appendix F) were also

provided to ensure that participants understood how to consume the oil properly. Furthermore, a checklist to keep track of VCO ingestion (Appendix H) was provided to each participant, and they were instructed that if they missed their dose one day, they could take double the following day. If they missed more than two days of supplementation (self-reported), their data would not be included in the study. All participants were tested within 24 hours of their final VCO dose.

3.4.2.5 – Day-to-Day Variability of the FMD Test

To validate the FMD test and the Cardiovascular Suite automated edge-detection software analysis technique employed in the present study, a test of day-to-day reliability was conducted by the principal investigator. Popliteal artery FMD tests were performed on 10 healthy participants, similar to the current study, on two separate days to determine if day-to-day differences exist.

Participants arrived to the lab in the same condition as outlined in the previous experiment (i.e. same time of day, hydrated, no confounding foods, etc.). Female participants also underwent their sessions during the same phase of their menstrual cycle (i.e. days 1-7, or placebo week).

Blood flow and artery diameter were recorded from the left PA slightly above the popliteal fossa (i.e. behind the knee) using a 12 MHz multi-frequency linear array transducer probe connected to a high-resolution ultrasound machine (Vivid i, General Electric Healthcare). The procedure used for the PA FMD was the same as outlined above in the previous experiment.

3.5 – Data Collection and Analysis

All ultrasound measurements were first recorded as videos (i.e. “cineloops”), on the Vivid i (General Electric Healthcare) ultrasound machine. Once ready for analysis, the cineloops

were transferred to a laptop computer via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa, Ont.) for offline analysis. The cineloops were analyzed using customized software (Cardiovascular Suite, Quipu), which were calibrated following the manufacturers recommendations, to determine changes (from baseline) in PA lumen diameter (mm), red blood cell velocity ($cm \cdot s^{-1}$), and shear rate (s^{-1}). A region of interest (ROI) of the PA image was selected and the customized software automatically traced the edges of the intima-lumen interface at the anterior and posterior vessel walls to continuously measure PA lumen diameter (mm) (Figure 3.3). To obtain red blood cell velocity, a ROI was selected around the pulsed-Doppler waveform signal and the customized software calculated the mean blood cell velocity across each heartbeat (Figure 3.3). The FMD tests were quantified as the percent change (from baseline) in peak diameter (FMD%).

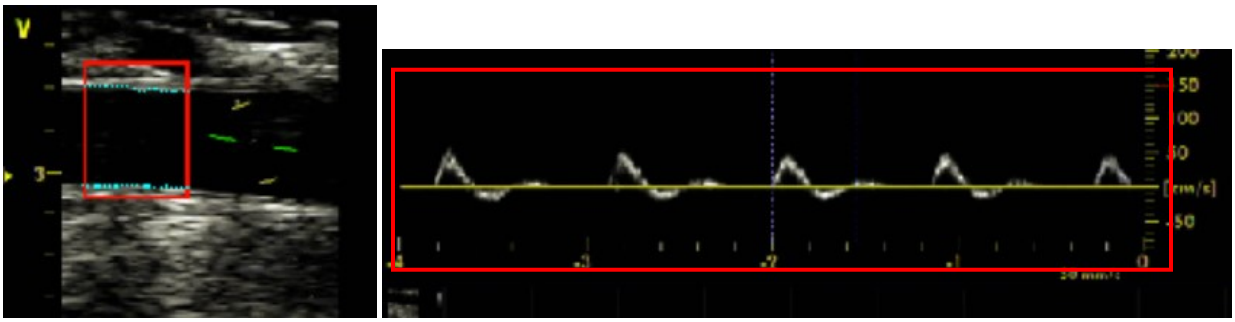

Figure 3.3: Examples of regions of interest selected around the lumen diameter, and the pulsed-Doppler waveform.

Resting PA lumen diameter (mm) was measured from the preceding baseline periods for the FMD and NTG tests. Peak PA lumen diameter (mm) was considered as the highest diameter measured during the five-minute post-cuff deflation or ten-minute post-NTG spray periods for the FMD and NTG tests, respectively. As before, FMD was quantified as the percent change (from baseline) in peak diameter (FMD%). Additionally, normalized FMD ($\%/SR_{AUC} \times 10^4$) was

calculated using the shear rate area under the curve (SR_{AUC}) from the time of cuff deflation to the time that peak PA diameter was achieved (Figure 2.23). NTG was represented as the percent change in diameter (NTG%) from baseline to the highest diameter that was attained during the ten-minute post-NTG spray period. Shear rate was calculated using the equation: Shear rate (s^{-1}) = (4 × mean red blood cell velocity, $cm \cdot s^{-1}$)/ PA diameter (mm).

Blood flow ($ml \cdot min^{-1}$) was calculated using the equation: blood flow ($ml \cdot min^{-1}$) = mean red blood cell velocity ($cm \cdot s^{-1}$) × π × (PA radius^2, cm^2) × 60 $s \cdot min^{-1}$. Resting PA red blood cell velocity ($cm \cdot s^{-1}$) and blood flow ($ml \cdot min^{-1}$) represent the average of the one-minute baseline period recorded on the cycle ergometer prior to the exercise test. Peak post-exercise red blood cell velocity ($cm \cdot s^{-1}$) was determined as the highest velocity recorded immediately following exercise. Post-exercise blood flow volume (ml) was determined as the area under the curve from peak post-exercise to five-minutes post-exercise.

The finger arterial pressure waveform obtained from the Portapres® (Finapres Medical Systems B.V, Amsterdam, The Netherlands) was sampled at 400 Hz and calibrated to the brachial artery blood pressure measurement collected using the automated blood pressure monitor (Carescape™ v100, General Electric Healthcare). This enabled collection of beat-by-beat recordings of systolic, diastolic, mean arterial (⅓ systolic + ⅔ diastolic), and pulse pressure (systolic – diastolic) by a data acquisition system (PowerLab, ADInstruments) using compatible software (LabChart, ADInstruments). These beat-by-beat recordings from the Portapres® waveform were also used to estimate values for cardiac stroke volume (SV; $ml \cdot beat^{-1}$) and cardiac output (Q; $L \cdot min^{-1}$) using the Modelflow® method in the dedicated Beatscope® analysis software (Finapres Medical Systems B.V, Amsterdam, The Netherlands). The Portapres® (Finapres Medical Systems B.V, Amsterdam, The Netherlands) data were also employed to

calculate total vascular conductance (TVC [ml·min^{-1}·mmHg^{-1}] = Q ÷ MAP) and leg vascular conductance (LVC [ml·min^{-1}·mmHg^{-1}] = Leg BF ÷ MAP) [174,175]. Additionally, age, sex, weight, and height of the participants was inputted into the model to calculate these variables accurately.

3.5.1 – Biochemical Analysis

A single blood sample was collected from each participant via finger prick both before and after VCO supplementation. A contact-activated lancet (BD Microtainer®) was used to puncture the capillary bed on the index finger following sterilization, and approximately 0.4 ml of blood was collected (drip method) into a 0.5 ml lithium heparin microcontainer (BD Microtainer®). Once collected, the blood samples were centrifuged at 10,000 G at 4°C for 15 minutes. The separated plasma layer was removed from the samples, aliquoted and stored at -20°C until assayed.

Total antioxidant capacity (US Biological) was measured using quantitative competitive ELISA, which utilized an anti-TAC antibody and a TAC-HRP conjugate. Microtiter plates were pre-coated with the anti-TAC antibody. Competition for limited antibody binding sites on the microplates occurred between TAC-HRP and TAC in the samples and standards. The assay protocol was followed according to the manufacturers' instructions, with a dilution factor of 1:4. Briefly, the plasma samples and TAC-HRP conjugate were added to the plates and incubated for an hour at 37°C. Following the incubation period the plates were washed five times and two substrates (A & B) were added to the wells. TAC concentration was measured spectrophotometrically at 450 nm using a microplate reader (BioRad Laboratories). The intensity of the resultant yellow colour was inversely proportional to the TAC concentration.

3.5.2 – Statistical Analyses

All data were expressed as means ± standard deviations, with a significance level set at $p < 0.05$. All statistical analyses were conducted using SPSS software version 22 (IBM). Normal distribution of all variables was determined through use of histograms and the Kolmogorov-Smirnov test.

To test the hypothesis that VCO increases PA function in healthy young adults, paired t-tests were used to compare the FMD and sublingual NTG-mediated dilation responses between pre- and post-VCO conditions. Paired t-tests were also used to assess differences in TAC concentrations and diet composition before and after VCO supplementation. A two-factor (condition × time) repeated-measures analysis of variance (ANOVA) was used to determine if there was a difference in PA blood flow either at rest or following moderate-intensity cycling exercise pre- and post-VCO ingestion. The assumption of sphericity was assessed using Mauchly's test, and a Greenhouse-Geisser correction to the degrees of freedom was applied for all significant tests.

The Bland-Altman technique [176] was employed to determine the level of agreement between the day-to-day repeated PA FMD tests. A one-sample t-test was used to determine fixed bias by testing the mean difference against zero. To determine proportional bias, linear regression analysis was performed to determine significant differences between the slope of the regression line and zero (results in Appendix G).

Chapter 4 – Results

4.1 – Resting Blood Pressure and Hemodynamic Data

19 participants (10 ♂, 22 ± 2 years, 24 ± 3 kg·m^{-2}) were included in this experiment. Participant resting characteristics are presented in Table 4.1. No differences were observed between pre- and post-VCO conditions for HR ($p = 0.10$), SBP ($p = 0.59$), DBP ($p = 0.34$), MAP ($p = 0.75$), Q ($p = 0.65$), or TVC ($p = 0.74$; Table 4.1). However, resting SV was lower ($p < 0.05$) following 4 weeks of VCO supplementation (Table 4.1).

Table 4.1: Resting blood pressure and hemodynamic data Pre- and Post-VCO supplementation.

	Pre-VCO	**Post-VCO**
HR (beats·min^{-1})*	67 ± 9	70 ± 8
SBP (mmHg)	116 ± 10	114 ± 12
DBP (mmHg)	58 ± 8	60 ± 9
MAP (mmHg)	77 ± 8	78 ± 9
SV (ml·beat^{-1})*	96 ± 14	89 ± 13†
Q (L·min^{-1})*	6.3 ± 0.9	6.2 ± 0.8
TVC (ml·min^{-1}·mmHg^{-1})*	74 ± 11	75 ± 9

Note: Data expressed as means ± standard deviation. VCO, virgin coconut oil; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SV, stroke volume; Q, cardiac output; TVC, total vascular conductance. *, n = 13; †, $p < 0.05$ vs. Pre-VCO.

4.2 – Popliteal Artery Endothelial-Dependent Dilation

Popliteal artery diameters both at baseline ($p = 0.26$) and at peak dilation ($p = 0.23$) were comparable in both conditions (Table 4.2). Following VCO supplementation, a significant increase ($p < 0.001$) in relative FMD (n = 19; Pre-VCO, 4.9 ± 0.9 %; Post-VCO, 5.7 ± 1.2 %, Figures 4.1A & 4.1B), but not SR_{AUC} (n = 11; Pre-VCO, 18.5 ± 5.8 s·10^{-3}; Post-VCO, 18.7 ± 4.7 s·10^{-3}, $p = 0.92$, Figures 4.1C & 4.1D), or FMD normalized to SR_{AUC} (n = 11; Pre-VCO, 3.1 ± 1.0 %/SR_{AUC}×10^{4}; Post-VCO, 3.3 ± 1.0 %/SR_{AUC}×10^{4}, $p = 0.18$, Figures 4.1E & 4.1F) was observed.

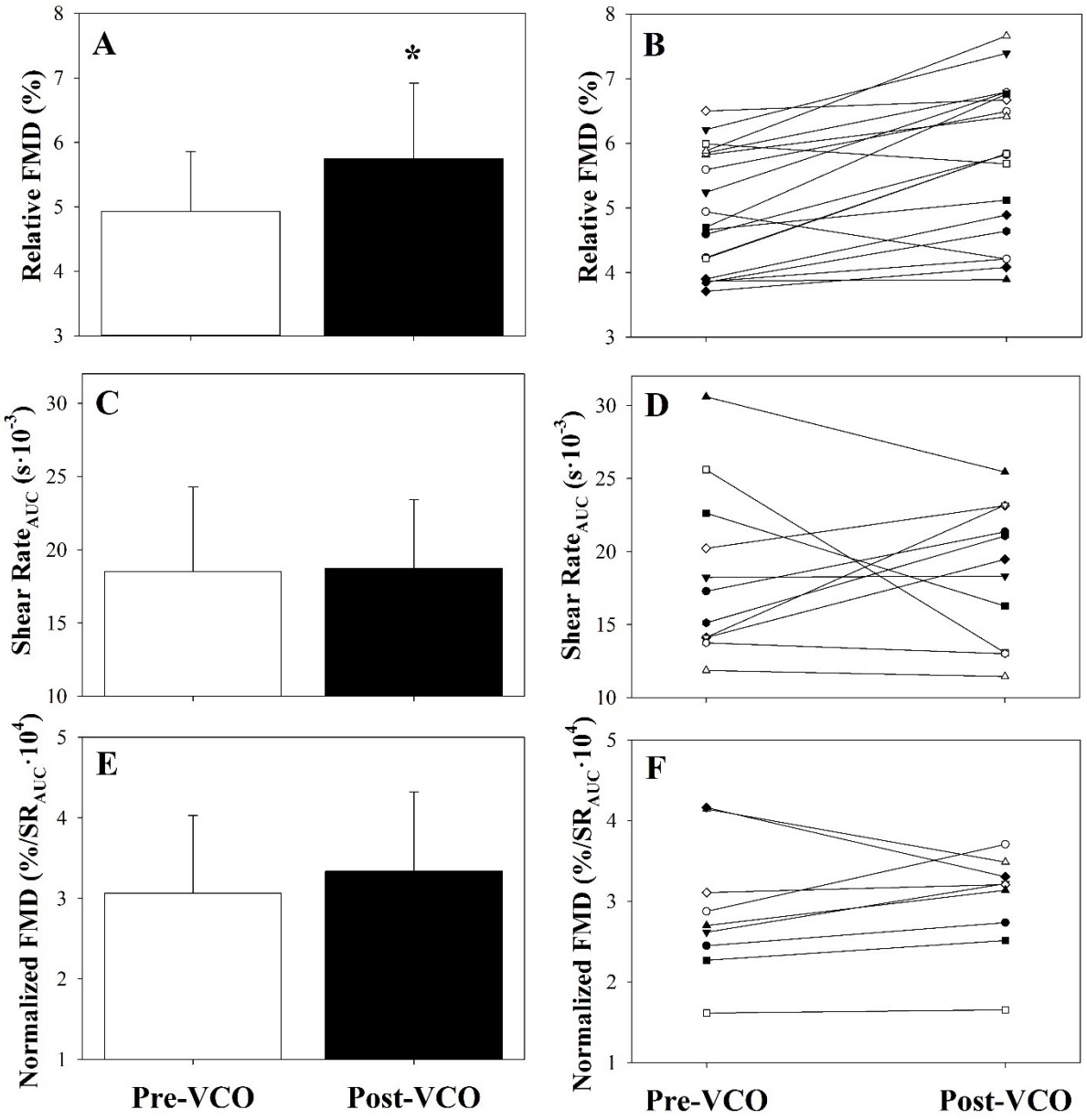

Figure 4.1: Whole sample and individual participant relative FMD (A, B), shear rate $_{AUC}$ (C, D), and normalized FMD (E, F) data before (white bars) and after (black bars) 4-weeks of VCO supplementation. Following VCO ingestion, significant enhancements were observed in relative FMD, indicating enhanced NO bioavailability. However, shear rate$_{AUC}$ and normalized FMD were not changed. Note, complete shear rate $_{AUC}$ and normalized FMD data were attained in 11 participants. *, $p < 0.001$ versus Pre-VCO. FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve; VCO, virgin coconut oil.

Table 4.2: Popliteal artery characteristics before and after 4-week VCO supplementation.

	Pre-VCO	Post-VCO
FMD		
Baseline Diameter (mm)	5.5 ± 0.8	5.3 ± 0.5
Peak Diameter (mm)	5.8 ± 0.8	5.6 ± 0.5
Time to Peak Diameter (s)	148 ± 48	135 ± 41
Baseline Shear Rate (s^{-1})*	57 ± 17	65 ± 19
Peak Shear Rate (s^{-1})*	261 ± 84	287 ± 101
Resting Flow ($ml \cdot min^{-1}$)	33 ± 28	32 ± 21
Leg Vascular Conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	0.49 ± 0.51	0.44 ± 0.27
NTG		
Baseline Diameter (mm)	5.6 ± 0.6	5.3 ± 0.5
Peak Diameter (mm)	6.1 ± 0.6	5.8 ± 0.6

Note: Data expressed as means ± standard deviation. All $p > 0.05$. VCO, virgin coconut oil; FMD, flow-mediated dilation; NTG, nitroglycerin. *, n = 11

4.3 – Biochemical Analysis

Plasma TAC was not significantly altered following a 4-week VCO supplementation in healthy young adults (Pre-VCO, 96.0 ± 15.1 $ng \cdot ml^{-1}$; Post-VCO, 96.8 ± 17.0 $ng \cdot ml^{-1}$; $p = 0.88$; Figure 4.2). One participant was excluded from the analysis due to excessive hemolysis of the sample. A five-parameter logistic regression standard curve was created to demonstrate the precision of the assay (Appendix I).

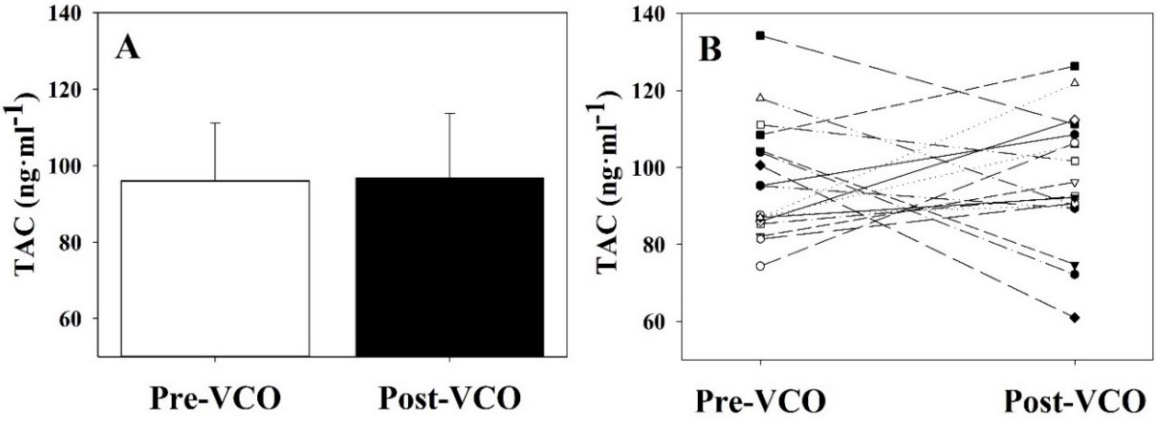

Figure 4.2: Whole sample (A) and individual participant (B) TAC ($ng \cdot ml^{-1}$, n = 18) data before and after 4-weeks of VCO supplementation. No change in group TAC was observed following VCO supplementation. However, TAC in 11 of 18 participants increased. TAC, total antioxidant content; VCO, virgin coconut oil.

4.4 – Popliteal Artery Endothelial-Independent Dilation

Virgin coconut oil supplementation did not alter either absolute baseline ($p = 0.053$) nor peak ($p = 0.065$) PA diameters during the NTG test (Table 4.2). There were also no differences in the relative change (NTG%) of PA lumen diameter (from baseline) following the sublingual NTG spray (Pre-VCO, 9.3 ± 2.5 %; Post-VCO, 9.9 ± 1.6 %; $p = 0.35$, Figure 4.3).

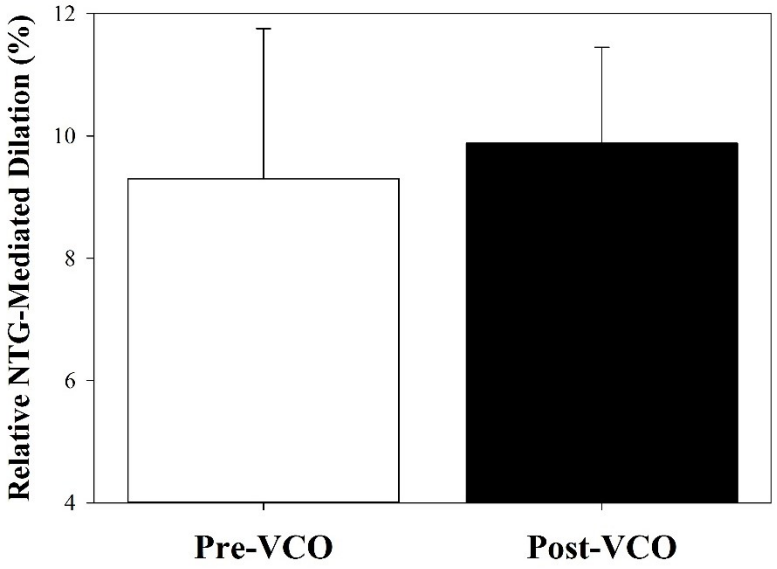

Figure 4.3: Relative NTG-mediated vasodilation (% baseline; n = 19) before and after 4-weeks of VCO supplementation. No significant difference in NTG-mediated dilation (i.e. VSM relaxation) was observed. NTG, nitroglycerin; VCO, virgin coconut oil.

4.5 – Popliteal Artery Exercise-Mediated Hyperemia

Pre-exercise PA diameter (Pre-VCO, 5.7 ± 0.8 mm; Post-VCO, 5.6 ± 1.1 mm) and blood flow (Pre-VCO, 38 ± 28 ml·min^{-1}; Post-VCO, 50 ± 24 ml·min^{-1}), were comparable (both, $p > 0.12$) between conditions. Additionally, VCO supplementation did not significantly enhance peak popliteal artery blood flow (Pre-VCO, 204 ± 145 ml·min^{-1}; Post-VCO, 228 ± 140 ml·min^{-1}; $p = 0.56$) or five-minute mean PA blood flow (Pre-VCO, 102 ± 75 ml·min^{-1}; Post-VCO, 124 ± 83 ml·min^{-1}; $p = 0.28$), and there was no main effect of the VCO supplement over the entire 5-minute post-exercise period ($p = 0.25$; Figure 4.4A). Five-minute post-exercise blood flow

volume was also not enhanced with VCO supplementation (Pre-VCO, 495 ± 355 ml; Post-VCO, 598 ± 384 ml; p = 0.28; Figure 4.4B).

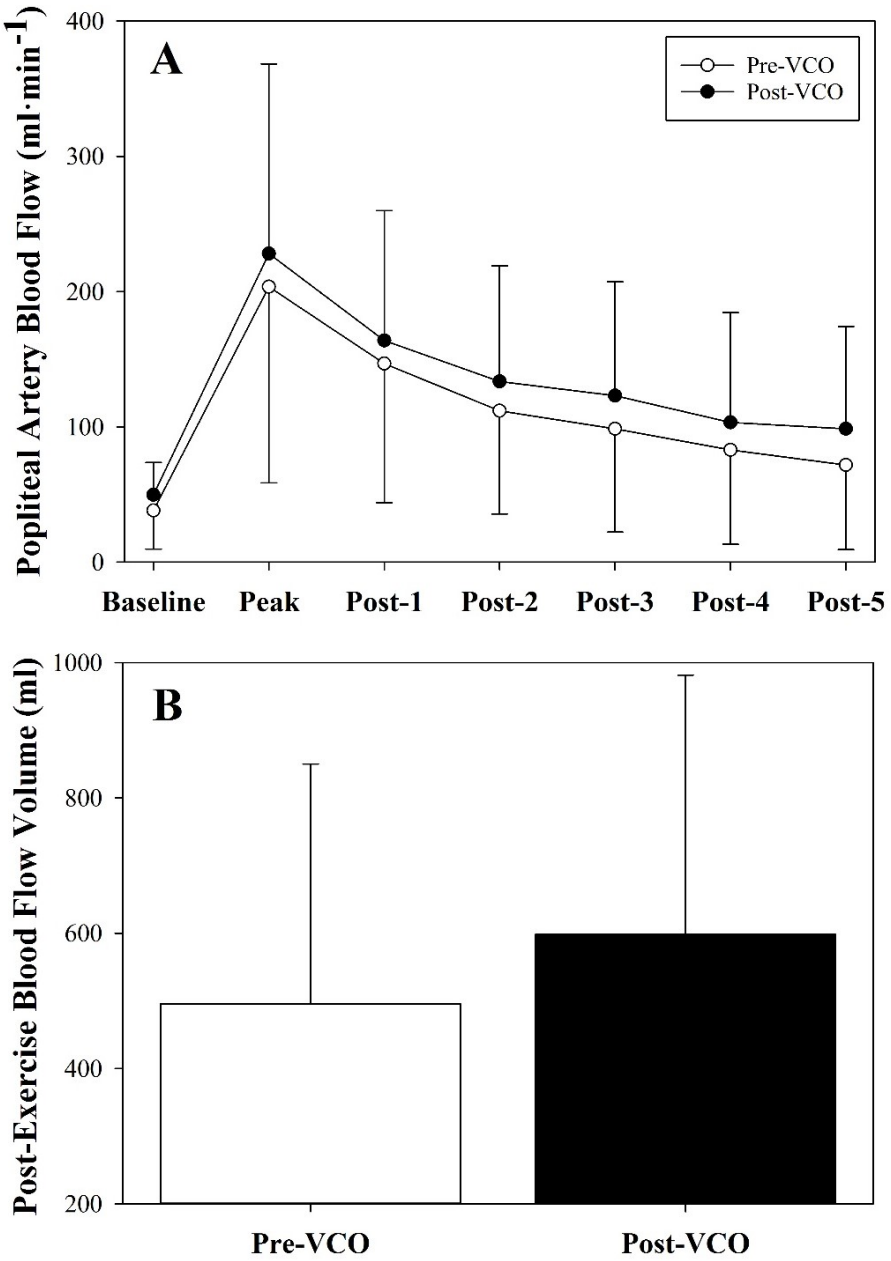

Figure 4.4: A) Popliteal artery blood flow (ml·min^{-1}) at rest, peak post-exercise, and for 5 minutes post-exercise (Post-1 to Post-5) and, B) five-minutes post-exercise popliteal artery blood flow volume (ml) before and after VCO supplementation (n = 19). No significant differences were observed following VCO ingestion. VCO, virgin coconut oil.

4.6 – Diet Composition

Aside from a significant decrease in meat & alternatives consumption (Pre-VCO, 41 ± 15 servings; Post-VCO, 33 ± 14 servings; $p < 0.05$), diet composition remained similar (all $p > 0.06$) before and after VCO ingestion (starches: Pre-VCO, 43 ± 15 servings; Post-VCO, 38 ± 9 servings ; fruits: Pre-VCO, 11 ± 9 servings; Post-VCO, 11 ± 9 servings; vegetables: Pre-VCO, 17 ± 7 servings; Post-VCO, 18 ± 9 servings; milk & alternatives: Pre-VCO, 7 ± 10 servings; Post-VCO, 8 ± 7 servings; fats: Pre-VCO, 17 ± 18 servings; Post-VCO, 18 ± 12 servings; sugary foods: Pre-VCO, 15 ± 12 servings; Post-VCO, 14 ± 12 servings; alcohol: Pre-VCO, 8 ± 10 servings; Post-VCO, 8 ± 9 servings; Figure 4.5).

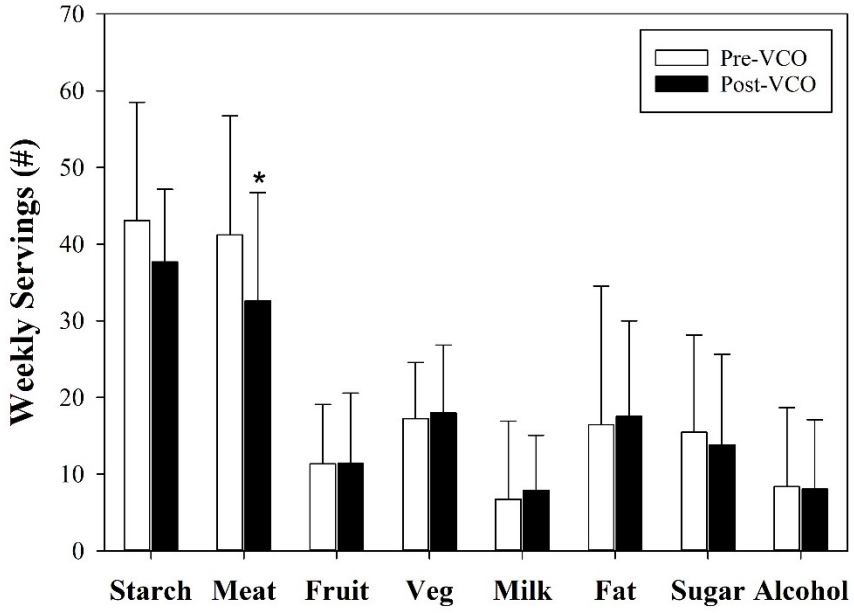

Figure 4.5: Weekly servings (#) of starches, meat & alternatives, fruits, vegetables, milk & alternatives, fats, sugary foods and alcohol. A significant decrease was observed in meat and alternatives during VCO ingestion. No significant differences in diet composition were reported for any other category throughout the study. VCO, virgin coconut oil. Meat, meat & alternatives; Veg, vegetables; Milk, milk & alternatives; Sugar, sugary foods. *$p < 0.05$ vs. Pre-VCO.

Chapter 5 – Discussion

In the present study, it was investigated whether a 4-week (30 ml·day^{-1}) ingestion of VCO in healthy young adults could enhance PA endothelial-dependent dilation (i.e. NO bioavailability), elevate the blood flow response induced by a single bout of moderate-intensity cycling exercise, and increase plasma TAC concentration. The results obtained demonstrate that 4-week VCO ingestion increased PA endothelial-dependent dilation, but not NTG-mediated dilation, exercise-mediated hyperemia, or plasma TAC levels.

5.1 – Resting Hemodynamic Data

Short-term VCO ingestion decreased SV by approximately 6% in healthy young adults. Stroke volume can be affected by several factors including preload (i.e. stretch of the heart), afterload (i.e. the pressure opposing ejection of blood), and contractility.[37] NO contributes to alterations in resting tone of the venous capacitance bed by increasing venodilation.[177] Changes to the venous system (i.e. increased venous relaxation) mediated by NO may have elicited a reduction in cardiac preload, therefore reducing SV. However, the change in SV was small (6%) and no corresponding decrease in Q was observed, due to a slight (non-significant) increase in HR. Therefore, other factors may have also contributed to the change observed in the present study.

The observed SV decline may have been mediated by a change in the exercise habits of the participants. Participants were asked to maintain their normal exercise regime for the duration of the study. However, this was not closely monitored. As little as two weeks of detraining (i.e. cessation of exercise) has been shown to reduce SV.[37,178,179] Therefore, if participants were exercising less than normal over the 4-week period (i.e. due to exams, busy

schedule, etc.), it is possible that SV would decline. Future research that controls for exercise (i.e. accelerometers) is required to fully understand the interaction between VCO and SV.

5.2 – Popliteal Artery Endothelial-Dependent Dilation

The PA FMD values obtained in the present study (Pre-VCO, 4.9 ± 0.9 %; Post-VCO, 5.7 ± 1.2 %) align with those from the repeatability analysis (Day 1, 5.0 ± 0.8 %; Day 2, 5.0 ± 0.6 %; Appendix H) and from previous studies investigating PA vasodilatory capacity in healthy young adults (4.4 ± 0.6% [180] to 6.1 ± 3.3% [155]). Normalized PA FMD in healthy young adults has been previously reported between 2 %/SR_{AUC}×10^4 [181] - 8 %/SR_{AUC}×10^4 [155,181] which also concurs with the current study (present study = Pre-VCO, 3.1 ± 1.0 %/SR_{AUC}×10^4; Post-VCO, 3.3 ± 1.0 %/SR_{AUC}×10^4).

The results of the FMD test align with the original hypothesis that VCO would increase the relative change in FMD. Although participant exercise habits were not recorded with physical activity monitoring devices, the improvement in endothelial function was not likely due to an increase in exercise training over the 4-week intervention period. The lack of change in other hemodynamic parameters (i.e. Q, HR, TVC), and a decrease in SV indicate that physical activity habits were not mediating the improvements in relative FMD. If exercise habits were increased to a level sufficient to improve endothelial function, these aforementioned hemodynamic parameters would have been increased as well.[37,178,179]

It is therefore probable that the improvement observed in NO bioavailability (i.e. FMD) was due to ingestion of VCO, and specifically, the antioxidant-activating polyphenol fraction. In animal studies that have demonstrated positive benefits from VCO administration, the typical quantity of VCO polyphenols ingested per day ranged from 0.07 to 1.56 $mg \cdot kg^{-1} \cdot day^{-1}$

(calculated using 84 mg polyphenols per 100 g; see section 2.6.2). This amount in a 70 kg human would range from 4.9 mg to 109.2 mg VCO polyphenols per day. Participants in the present study ingested approximately 27 g (equal to 30 ml) of the oil per day, which means approximately 22.7 mg of polyphenols were consumed per day. Therefore, if it is possible that positive health benefits could be observed with a small quantity of polyphenols (i.e. 4.9 mg), then it is reasonable that 22.7 mg of polyphenols per day could elicit a significant increase in PA FMD.

It is unclear whether the influence of VCO on NO bioavailability observed in the present study was mediated by the polyphenol fraction. Before now, the only literature investigating this interaction were conducted in animals.[28,32] However, numerous reports of improvements in human endothelial function following ingestion of other polyphenols may support this theory.[15,182–186] The effects of grape polyphenols (i.e. anthocyanins, flavanols, flavonols, proanthocyanidins, and stilbenes) on brachial artery FMD have been most extensively researched.[183,184,187] Blumberg et al.[183] conducted a review of clinical trials that assessed the impact of 100% Concord grape juice (acute, 355-710 ml) on biomarkers of CVD risk (i.e. brachial artery FMD, and the resistance of LDL to oxidation). It was found that all concentrations of the Concord grape polyphenols elicited beneficial effects in both risk factors (FMD increased ~2%; lag time of LDL to oxidation increased ~16-19%). Another group [184] conducted a meta-analysis on the effects of an acute dose (600-1200 mg) of grape polyphenols on brachial artery FMD in healthy adults. It was also concluded that endothelial function can be significantly improved (~1-3 %) following an acute intake of grape polyphenols, and that this improvement was likely mediated through an increase in NO bioavailability.

A second group of polyphenols commonly shown to improve endothelial function are chlorogenic acids (i.e. coffee bean polyphenols).[182,185,186] Ward et al.[182] divided 16 healthy men (18-70 years) into 4 groups (0 mg, 450 mg and 900 mg chlorogenic acids and 200 mg epicatechin as a positive control) to assess the impact of an acute dose of chlorogenic acids on brachial artery FMD, and BP. Both low- and high-doses of chlorogenic acids did not change BP, or peak FMD, but did significantly improve the one-hour post-ischemic FMD response (0.4 – 0.8 %). Other studies similarly concluded that acute doses (600 mg [186] and 335 mg [185]) of chlorogenic acids were able to improve brachial artery FMD (~1.5% and 1%, respectively) in healthy men (24-53 years).

Recently, in 14 healthy individuals (20-40 years), Sanguigni et al.[15] assessed the impact of an acute dose of polyphenol-rich natural antioxidant ice cream (100 g; polyphenol concentration not available) versus control (100 g milk chocolate ice cream) on nitric oxide bioavailability (represented by presence of nitrite and nitrate metabolites), brachial artery FMD, and reactive hyperemia index. The reactive hyperemia index was measured using EndoPat and test signal [T] relative to baseline [B] signal (i.e. post-occlusion until return to baseline). Two hours post-prandial, the polyphenol-rich ice cream significantly increased NO metabolites (control ~27 ± 3 mM vs. ice cream ~42 ± 5 mM), brachial artery FMD (control ~2.0 ± 0.5 % vs. ice cream ~6.3 ± 1 %) and reactive hyperemia index (control ~3.4 ± 0.6 T/B, ice cream ~ 4.5 ± 1 T/B).

Overall, polyphenol-rich foods and supplements appear to improve endothelial function through an increase in NO bioavailability (i.e. FMD). In the present study, VCO, which contains a relatively smaller dose of polyphenols (i.e. 22.7 mg), significantly enhanced PA FMD (~0.5-

1%). These results collectively indicate that the polyphenols present in VCO are likely responsible for the enhancement in FMD observed in the present study.

There is also potential that isolating the polyphenol fraction of VCO would improve endothelial function even further in healthy humans. Very recently, the American Heart Association issued a statement indicating that coconut oil raises LDL-C concentrations in humans [188] due to its high concentration of saturated fats.[136] It was concluded that coconut oil may increase CVD risk and should therefore be replaced with unsaturated fats. Low-density lipoprotein cholesterol particles can disrupt endothelial function and NO bioavailability.[89] Therefore, removal of the saturated fat portion of VCO, in conjunction with the action of the polyphenol fraction, may improve endothelial function further. Obtaining an isolated dose of VCO polyphenols without the high saturated fat content may be an important next step in endothelial health research.

5.3 – Biochemical Analysis

Although FMD was increased, TAC was not significantly altered following 4-week VCO supplementation. However, there are some potential explanations for these results. Previous studies that investigated whether high concentrations of polyphenols can increase FMD also assessed for polyphenol and antioxidant content of the plasma.[15,182] Acute doses of chlorogenic acids (450 mg and 900 mg) were found to significantly increase the chlorogenic acid metabolites of the plasma at 1 (control 0.0 μM; 450 mg ~0.7 μM; 900 mg ~1.4 μM) and 4 hours (control 0.0 μM; 450 mg ~0.9 μM; 900 mg ~1.1 μM) following ingestion.[182] Additionally, when compared to a control, two hours post-prandial, polyphenol-rich ice cream (100 g) significantly increased serum polyphenols and antioxidant status (control ~160 ± 35 mg·L vs. ice cream ~270 ± 30 mg·L GAE^{-1}) while decreasing oxidative stress (i.e. plasma hydrogen peroxide concentration)

(control 1.6 ± 0.2 mM vs. ice cream ~ 1.1 ± 0.1 mM).[15] It is therefore possible that higher concentrations of VCO polyphenols (i.e. > 100 mg) than those occurring naturally in the present dose of VCO (i.e. 22.7 mg) are needed to elicit a change in TAC content of the plasma. Additionally, contrary to the present study where the final dose of VCO was ingested the day before testing, all experiments that demonstrated an increase in plasma polyphenols administered an acute dose of polyphenols 1-6 hours prior to blood collection. This may indicate that high concentrations of polyphenols are present in the plasma shortly after administration, but not maintained in the plasma over time.[104]

However, despite these results, it was found that most participants (11 of 18; one excluded) demonstrated an increase in TAC following 4-week VCO ingestion (Figure 4.5B). When a paired t-test was performed on these participants alone, VCO was found to significantly increase TAC (Pre-VCO, 87.3 ± 8.6 $ng \cdot ml^{-1}$; Post-VCO, 103.9 ± 12.7 $ng \cdot ml^{-1}$; $p < 0.001$). It is therefore possible that increasing the sample size could elicit a significant increase in plasma TAC following VCO ingestion. It should be noted that no correlation ($p = 0.73$) was observed between the improvement in FMD% and increase in TAC concentration. However, another interesting point to note is that 9 of 10 males, but only 2 of 8 females were included in this responsive subgroup. Therefore, there is potential that TAC in certain individuals may increase more than in others, though this does not appear to be related to endothelial-dependent dilation.

5.4 – Popliteal Artery Endothelial-Independent Dilation

The PA endothelial-independent dilation results obtained in the present study are also comparable to those from previous research. Peak PA NTG values typically range from 6.5 ± 0.9 mm [144] to 6.7 ± 0.9 mm [155] (present study = Pre-VCO, 6.1 ± 0.6 mm; Post-VCO, 5.8 ± 0.6 mm),

and relative changes in PA NTG range from 8.5 ± 3.8% [155] to 9.1 ± 3.5% [144] (present study = Pre-VCO, 9.3 ± 2.5%; Post-VCO, 9.9 ± 1.6%).

As expected, no significant increase in endothelial-independent dilation was observed in the present study. Nitroglycerin acts as a NO-donor, bypasses the endothelium, and acts directly on the VSM to elicit maximal relaxation. The NTG test provides an index regarding the sensitivity of VSM cells to dilate in response to NO. If VCO directly influences endothelial function, a test of dilation independent of the endothelium should not be affected by VCO ingestion.

Similar to endothelial-dependent dilation, the effect of VCO polyphenols on NTG-mediated dilation, has not been investigated. However, information pertaining to the influence of other polyphenols on NTG-mediated dilation is available. Djoussé [115] assessed whether a single high-fat meal with red wine (i.e. polyphenol-containing), or with an isocaloric amount of Coca-Cola (3 $ml \cdot kg^{-1}$ body weight) affected the vasodilatory response to nitroglycerin in healthy individuals (32 ± 9 years). It was found that the vasodilatory response to nitroglycerin was not affected by the polyphenol-containing beverage.[115] Choi et al.[189] investigated whether 100 $mg \cdot day^{-1}$ onion peel polyphenols (i.e. quercetin) for 12 weeks was able to improve endothelial-dependent (i.e. brachial artery FMD) and independent dilation (i.e. nitroglycerin) in healthy individuals (43 ± 9 years; 26.8 ± 3.2 $kg \cdot m^{-2}$). Similar to the present study, it was found that the polyphenols improved FMD after 12 weeks (from 12.5 ± 5.2 % to 15.2 ± 6.1 %) however nitroglycerin-mediated dilation did not change. These results support the likelihood that short-term ingestion of VCO is unable to enhance NTG-mediated dilation further in healthy young adults.

5.5 – Popliteal Artery Exercise-Mediated Hyperemia

The resting PA blood flow values obtained in the present study (Pre-VCO, 38 ± 28 $ml \cdot min^{-1}$; Post-VCO 50 ± 24 $ml \cdot min^{-1}$) align with those from previous studies investigating PA exercise-mediated hyperemia in healthy young males (22-31 years).[171,190] In these studies, resting PA blood flow values ranged from 48 ± 28 $ml \cdot min^{-1}$ [190] to 52 ± 15 $ml \cdot min^{-1}$.[171]

Although VCO increased FMD, no significant increase in exercise-mediated hyperemia was observed. This may indicate that NO does not play as significant a role in exercise-mediated hyperemia as previously believed. In agreement with the present study, it was recently found that there is only a minor contribution of NO to the blood flow response during dynamic exercise (5 minutes of 15% maximal handgrip exercise).[191] Limberg et al.[191] divided healthy young adults (25 ± 1 years) into four separate groups, including a control condition (i.e. no drugs) and three experimental conditions:1, oral sildenafil citrate (inhibitor of phosphodiesterase-5, an indirect vasoconstrictor); 2, intra-arterial L-NMMA (NO inhibitor); 3, oral sildenafil citrate and intra-arterial L-NMMA). Phosphodiesterase-5 dissociates cGMP into 5-guanosine monophosphate and inhibits vasodilation of the VSM [2] (see Figure 2.7). Blood flow was determined through Doppler ultrasound. Infusion of L-NMMA resulted in ~12% reduction in exercise hyperemia but sildenafil citrate did not elicit an additional effect. The authors therefore concluded that although their observations reflected a contribution of NO and the cGMP pathway during exercise hyperemia, this contribution was smaller than previously believed (~15% vs. ~20-30% [192]). To further support the notion that NO only contributes a small amount to exercise hyperemia, Shabeeh et al.[193] assessed the impact of eNOS and neuronal NOS inhibition on forearm blood flow at rest, during sympathetic activity by lower body negative pressure, and during lower body negative pressure immediately after handgrip exercise in healthy males (26 ± 7 years). Inhibition

of eNOS was mediated by L-NMMA, and inhibition of neuronal NOS was mediated by selective inhibitor *S*-methyl-L-thiocitrulline. Reductions in forearm blood flow by lower body negative pressure were increased by L-NMMA administration, but not *S*-methyl-L-thiocitrulline, indicating that eNOS compensates in some part for elevated resting vasoconstriction. However, following exercise, there were no additional reductions in forearm blood flow observed with either L-NMMA or *S*-methyl-L-thiocitrulline, potentially indicating a less essential role of NO in mediating the post-exercise hyperemic response.[193]

In agreement with these previous studies, Joyner & Casey [194] conducted a review in which the various contributing factors to exercise-mediated hyperemia were extensively outlined. It was concluded that NO only contributes to approximately 15% of the hyperemic response to exercise. Therefore, it is conceivable that VCO would significantly increase FMD, which is primarily NO mediated, and not significantly increase exercise-mediated hyperemia as was demonstrated in the present study. Furthermore, as noted in section 2.3, exercise hyperemia is mediated by a variety of factors (e.g. local metabolites, myogenic autoregulation, muscle pump, etc.), therefore these factors likely contribute more to the hyperemic response than NO.

5.6 – Diet Composition

Participant diet composition (i.e. # of servings) remained consistent throughout the study, except for meat & alternatives intake. Endothelial function and arterial reactivity may be affected by an abnormal protein intake.[195,196] A normal protein intake, but not a high protein intake, improves endothelial function (i.e. brachial artery FMD) in healthy men (26 ± 5 years) by approximately 4%.[195] Conversely, when compared to a control group (18% casein), a low-protein intake (9% casein) has been shown to reduce vascular relaxation, determined through acetylcholine-induced relaxation of dissected branches of the mesenteric arteries, in virgin and

pregnant rats by approximately 4%.[196] Therefore, it is possible that the lower intake of meat & alternatives following short-term VCO ingestion could have reduced arterial function of the participants.

However, in the current study, no significant decrease in vascular relaxation was observed (i.e. FMD and NTG-mediated dilation), and endothelial function was enhanced (i.e. FMD) following short-term VCO ingestion. Protein is abundant in many other dietary sources (i.e. vegetables, fruit, dairy products).[197,198] Therefore, it is possible that the decline in meat & alternatives consumption did not reflect a significant decrease in protein intake. The choice to record diet as serving sizes was made to provide participants with a simplified diet breakdown that they could follow for the duration of the study. However, future research should also include a macronutrient (i.e. carbohydrates, fats and proteins) assessment to ensure that diet remains as consistent as possible, and to prevent any adverse consequences to arterial function.

5.7 – Limitations

The most significant limitation of this study is that a placebo/control group was not used. We decided to implement a pre/post study design for feasibility, because this was the first study to assess the impact of VCO supplementation on vascular endothelial function. We compared the post-VCO measurements of each participant to their baseline measurements (control). To decrease any confounding variables, we tested all participants at the same time of day, we tested all females at the same stage in their menstrual cycles, and we asked participants to keep a diet log and maintain a consistent diet throughout the study. Participants were also given guidelines regarding specific activities and foods to avoid that could influence the FMD test. Furthermore, the results of our day-to-day reliability analysis indicated that no bias was present in our FMD

procedure. However, deciding to exclude a placebo group decreased the power of the study, and increased the chance of error. Therefore, it is recommended that future research should include a placebo group.

Another limitation of this study was that we did not evaluate additional plasma markers commonly assessed in VCO research, such as plasma polyphenols, NO metabolites (i.e. nitrite and nitrate), ROS (i.e. superoxide), LDL-C, HDL-C, or triglycerides.[188] Assessing for these variables could have provided us with more information on the antioxidant capacity of VCO in healthy adults. It may also have provided support for our hypothesis that VCO polyphenols were responsible for the increase in FMD%. Furthermore, with the new recommendations from the American Heart Association,[136] it is now important to assess for LDL-C concentration, as this may indicate a health risk. Additionally, we did not measure waist-to-hip ratio, a variable from the studies on which we based our dosage. This would have allowed us to confirm whether additional health effects of VCO exist.

5.8 – Future Recommendations

This project is important because it is the first to evaluate the interaction between VCO and vascular function in humans. We sought to advance the state of knowledge surrounding vascular function, and inspire future research in VCO and CVD risk prevention. A goal of the current study was, and still is, to change opinions on VCO use and modify its Health Canada classification to a food or natural health product. Virgin coconut oil is a natural food product, meaning its production does not involve the use of preservatives, or harmful chemical refining. It is easy to use, delicious to eat, and widely available for a reasonable cost in most major grocery stores in North America. It may be added to a variety of foods such as smoothies or desserts, and may be cooked with as an alternative to butter or other oils.

However, as mentioned previously, the American Heart Association has recently released a report expressing their concern with widespread use of coconut oil due to its high saturated fat content.[136] Therefore, replacing butter and other fats with coconut oil may not be feasible. An alternative solution for the future could be to isolate the polyphenol fraction of VCO, and assess its impact on endothelial function and other CVD risk factors. Furthermore, isolating the polyphenols could help with participant and consumer compliance, because some participants commented that ingesting 30 ml VCO per day became cumbersome, and they would not continue to eat this quantity after the study.

We also advise that the effect of VCO on endothelial function be examined in persons of different ages and disease states before definitive recommendations are made. It would be interesting to see if individuals with impaired vascular endothelial function (i.e. older adults,[199] hypertensive patients,[200] CVD populations [3]) would experience greater benefits following supplementation with VCO. Moreover, it has been demonstrated that ROS play a key role in the aging cardiovascular system, partly by inducing excessive infiltration of VSM cells into the sub-endothelial space.[201] This infiltration contributes to stiffening of the arteries, and prevents maximal relaxation of the VSM.[201] VCO polyphenols, which stimulate the action of ROS-scavenging antioxidants, could potentially play a long-term role in CVD prevention and may eventually elicit a significant change in NTG-mediated dilation (i.e. VSM reactivity). This may be an interesting avenue to explore in future research.

5.9 – Conclusion

Overall, short-term VCO ingestion increased PA endothelial-dependent dilation, but was unable to augment exercise-mediated hyperemia or endothelial-independent dilation. These observations may reflect both an ability of VCO to increase NO bioavailability, and a relatively

lower contribution of NO to the exercise-mediated hyperemic response. VCO ingestion also failed to increase mean plasma TAC. However, plasma TAC of 11 of 18 participants was significantly enhanced, indicating possible benefits with a larger sample size. Future research should consider the effect of VCO supplementation on the endothelial function of populations at risk for CVD. Furthermore, it may be prudent to assess the impact of an isolated solution of VCO polyphenols in order to comply with the new recommendations of the American Heart Association.

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Appendices

Appendix A: Recruitment Poster

INTERESTED IN KNOWING IF **VIRGIN COCONUT OIL** CAN IMPROVE YOUR BLOOD VESSEL FUNCTION?

You may be eligible to participate in this study if you are:

- **Between the ages of 18-30 years**
- **Free of chronic disease**
- **BMI < 30**
- **Not pregnant or breastfeeding**

Duration: 3 visits, total ~4 hours (modest remuneration provided)

We will take a small blood sample from the index finger and use an ***ultrasound machine*** to measure the size and blood flow in a lower leg artery, at rest, after restriction of blood flow, after ingestion of nitroglycerin (a substance to relax the artery), and following 10 minutes of moderate-intensity cycling exercise.

What will you need to do?

- Take 2 tbsp. virgin coconut oil per day for four weeks
- Maintain a food journal for two separate weeks

School of Health and Human Performance – Dept. of Kinesiology

VIRGIN COCONUT OIL STUDY Susan Robinson susan.robinson@dal.ca	**VIRGIN COCONUT OIL STUDY** Susan Robinson susan.robinson@dal.ca	**VIRGIN COCONUT OIL STUDY** Susan Robinson susan.robinson@dal.ca	**VIRGIN COCONUT OIL STUDY** Susan Robinson susan.robinson@dal.ca	**VIRGIN COCONUT OIL STUDY** Susan Robinson susan.robinson@dal.ca	**VIRGIN COCONUT OIL STUDY** Susan Robinson susan.robinson@dal.ca	**VIRGIN COCONUT OIL STUDY** Susan Robinson susan.robinson@dal.ca	**VIRGIN COCONUT OIL STUDY** Susan Robinson susan.robinson@dal.ca

Appendix B: Information Letter and Consent Form

CONSENT FORM

Project Title: Can short-term ingestion of coconut oil improve vascular function and exercise-mediated hyperemia in healthy young adults?

You are invited to take part in a research study being conducted by Susan Robinson who is a graduate student at Dalhousie University, as part of her Master's thesis in Kinesiology. Ms. Robinson is supervised by Dr. Derek Kimmerly, an Assistant Professor of Kinesiology at Dalhousie University, School of Health and Human Performance. Your participation in this study is voluntary and you may withdraw from the study at any time. There will be no impact on your studies or academic evaluation if you decide not to participate in the research. The information below tells you about what you will be asked to do and about any benefit, risk, or discomfort that you might experience.

Who will be conducting the research?

The Principal Investigators will be Susan Robinson and Dr. Derek Kimmerly from the School of Health and Human Performance, Division of Kinesiology at Dalhousie University. Susan Robinson, M.Sc. Kinesiology candidate, will recruit participants and conduct the research as part of her Master's research thesis. Dr. Kimmerly can be contacted via email (dskimmerly@dal.ca) or telephone at (902) 494-2570 should you have any questions or concerns about the study.

Purpose and Outline of the Research Study

Virgin coconut oil has become increasingly popular as a healthy food product, and has recently been recognized in animal studies for its potential in the protection of the cardiovascular system. Virgin coconut oil is different from regular coconut oil because when it is extracted, it retains all of its vitamins and antioxidants. The antioxidants in virgin coconut oil have been suggested to improve blood vessel function, and enhance the production of nitric oxide. Nitric oxide helps to relax blood vessels and increase blood flow at rest and during exercise.

Much of the research conducted to date has been in animals, therefore the purpose of this study is to investigate the potential benefits of virgin coconut oil on blood vessel function in humans.

Who Can Participate in the Research Study?

You may participate in this study if you are between 18-30 years old and do not have a chronic condition or disease that affects the heart or blood vessels.
You may not participate in this study if you:

- Have a resting blood pressure >140 mmHg (systolic) or >90 mmHg (diastolic). We can measure this for you.
- Are pregnant or breastfeeding.
- Have smoked cigarettes or consumed any nicotine containing product within the past 6 months.
- Have been diagnosed with a medical condition that affects your cardiovascular system.
- Have been told not to engage in strenuous physical activity by a physician.
- Have answered 'yes' to any questions on the Physical Activity Readiness-Questionnaire.
- Suffer from joint problems or other physical limitations that will not permit you to exercise.
- Have a body mass index > 30 kg/m^2. Please see the 'Health History Questionnaire' to determine how to estimate your body mass index.
- Have an irregular menstrual cycle (i.e. do not menstruate consistently between days 1-7 of a 28-day cycle).

What You Will Be Asked to Do?

The study will involve a total of 3 visits and approximately 4 hours of your time.
On Day 1 of testing (approximately 1 hour), we will ask you to complete some questionnaires, measure your height, weight and blood pressure (upper left arm), provide a tour of the laboratory and explain all of the equipment and procedures used for the study. Once you are deemed eligible to participate in the study we will ask you to perform a submaximal cycling exercise test. We will ask you to please bring clothing you are comfortable exercising in. We will have you affix your own wireless chest strap heart rate monitor prior to exercising. The cycling test will involve having you rest for a couple of minutes on the cycle seat to measure your resting heart rate. We will set the resistance on the cycle to a low level so you can 'warm-up' for 5 minutes. The intensity will then increase every 3 minutes until you reach a target heart rate that is about 60% of your heart rate reserve. We will record the seat height and intensity for use during a 10-minute exercise session on Days 2 and 3 (see below).

Prior to leaving at the end of Day 1, you will be given a daily food journal and asked to record your dietary intake for one week prior to Day 2. Additionally, you will be asked to abstain from consuming antioxidant supplements, such as vitamin C or E, CoQ-10 (ubiquinone), alpha-lipoic acid, grape seed extract (resveratrol), carotenoids, green tea extract, L-glutathione, and quercetin for the remainder of the study.

On Day 2 of testing (approximately 1.5 hours), we will take a single blood sample from you to assess for free radical and antioxidant content of your blood using the finger prick method. We

will also measure your resting heart rate, blood pressure and lower leg blood flow while you rest quietly lying on your left side or stomach. Heart rate will be recorded using the same wireless monitor as Day 1. Blood pressure will be determined from your upper left arm using an automatic vital signs monitor and from an index or middle finger using a small pressure cuff. Blood flow will be measured using an ultrasound machine in an artery behind your right knee. As such, we will ask you to please bring clothing you are comfortable in, preferably that expose your lower leg or can be rolled up. We will record these signals for a minimum of 5 minutes while you remain lying down on the lab bed.
We will then perform a test to provide an indication of how much nitric oxide the artery in your lower leg can make. A pressure cuff will be placed over the middle of your right calf below where we are measuring blood flow. The cuff will quickly be inflated to a level that will stop blood flow to your calf and foot and remain inflated for 5 minutes. This period of inflation may cause some discomfort. The cuff will then quickly be deflated and blood flow will return. During the cuff inflation and for an additional 5 minutes after the cuff pressure has been released, we will continue to measure blood flow and view images of your artery using the ultrasound machine.

We will let your rest for 10 minutes to allow the blood flow in your artery to return to resting levels. We will then examine how your artery relaxes after a single spray of nitroglycerin underneath your tongue. We will view images of your artery and measure blood flow for 10 minutes after you are given the nitroglycerin spray.

You will then move to the same bicycle used in your first visit. After a 5-minute warm-up, we will increase the resistance to the level that was determined on Day 1. You will continue to cycle for 10 minutes at this intensity. Small changes in resistance may be made if your target heart rate gets higher or lower than expected. We will then directly measure your lower leg blood flow for 5 minutes while you remain seated on the exercise bike, as well as your heart rate and blood pressure. Once your blood pressure has returned to pre-exercise levels your test will be complete.

At the end of Day 2 we will provide you with the 4-week supply of virgin coconut oil. You will be instructed to consume two pre-measured tablespoons (alone or with food such as a smoothie or a pre-cooked stir-fry – no cooking with the oil will be permitted as this may affect the antioxidant content of the oil), one tablespoon at breakfast, and one at dinner during the four weeks prior to your last visit (Day 3), while continuing to avoid the antioxidant supplements mentioned previously. Additionally, based off of your daily food journal, you will be provided with information concerning your average intake of protein, carbohydrates, and fats, and you will be asked to maintain this intake for the four weeks leading up to Day 3. You will also be provided with an additional daily food journal to record your dietary intake for one week prior to

Day 3. During Day 3 (approximately 1.5 hours) we will repeat the above procedures outlined for Day 2.

Possible Benefits, Risks and Discomforts

You will receive a small monetary benefit for participating in this study. Additionally, information regarding your general cardiovascular health including resting blood pressure, heart rate, and lower leg blood flow will be provided to you upon request.

Moderate-Intensity Cycling Exercise: Performing moderate-intensity exercise is safe but can induce unpleasant side effects such as sweating, an elevated heart rate, shortness of breath, dizziness, and light-headedness. These feelings will start to decrease once exercise stops. First aid qualified personnel will ensure your safety by helping you manage these symptoms if they arise. We will have the ability to call for medical assistance if necessary.

Heart Rate Measurements: You will place a chest strap wireless monitor around your upper chest that will record the electrical activity of your heart. Redness may develop on the skin after you remove the strap but should disappear within a few hours.

Finger Blood Pressure Measurements: You may experience slight discomfort as a result of these measurements, including sensations of “pins and needles” in your fingers. When in use, the cuffs will inflate with air and you should feel it gently squeeze your fingers. Your fingers may turn slightly blue and feel numb or tingly when this cuff is inflated. The symptoms go away once the cuff pressure is reduced. Some people may feel slight pain in this procedure. Pain is not expected to be any worse than what you would feel when a physician takes your blood pressure. If you have felt pain due to blood pressure cuffs in the past, you may not want to take part in this study.

Automated Upper Arm Blood Pressure Measurements: Slight discomfort may be experienced by the participant as the blood pressure cuff is inflated, which may or may not include feelings of "pins and needles" in the participant's hand. This sensation should be relieved as soon as the cuff has been deflated.

Blood Sample via Finger Prick: Obtaining a blood sample through a finger prick will cause some slight pain in the immediate area. Puncture to the skin will be minimal, so bleeding should cease quickly if not immediately after the required drops of blood are obtained. You will be provided with Band-Aids® if necessary to control bleeding and provide pain relief through slight pressure.

Ultrasound measurements of leg blood flow: There are minimal risks (<5%) associated with the transmission of high-frequency sound waves into the body. With prolonged scanning, the skin

may become slightly red and warm. These effects will quickly dissipate once the ultrasound probe has been removed.

Tests of Blood Vessel Function: How your popliteal artery (located behind your knee) responds to a stimulus will be measured by taking pictures of it with an ultrasound. The ultrasound is non-invasive with minimal risks. In rare cases, you may experience a slight rash from the ultrasound gel. There is no known medical risk associated with a 5-minute period of blood flow stoppage to one's leg, and the technique has been used on thousands of people worldwide. Nevertheless, during the 5-minute no-flow period you may feel numbness, a cold sensation, tightness and there may be a slight bluing in foot or lower leg colour. All of these symptoms will go away once we have released the cuff after 5 minutes.

The reported possible side effects of nitroglycerin administration include: dizziness, lightheadedness, or fainting when sitting up or standing, headache, rash, nausea or vomiting, fast heartbeat, or flushing of the face and neck. The most likely side-effect will be dizziness or lightheadedness. These side effects occur in less than 5% of people. However, the administration of nitroglycerin while you are lying down should minimize this risk. If, however, dizziness or lightheadedness does occur, it should quickly subside. We will measure your blood pressure after nitroglycerin administration and ask you to remain lying down until your blood pressure is at the same level as it was before the nitroglycerin spray was provided.

Steps have been taken to ensure that all procedures will be performed with minimal risks of any adverse health effects. Throughout the study we will be recording numerous measures of your health. If for any reason we find information that may show a possible health risk (e.g. high resting blood pressure), we will explain the issue to you and strongly recommend that you visit your family doctor. If this occurs, you may no longer be eligible to participate in the study.

Compensation / Reimbursement

You will be compensated $40 for completion of this study. Twenty dollars will be provided following the completion of Day 2 with another $20 provided following the completion of Day 3. In addition, we will also reimburse you for any transportation-related expenses (e.g. parking, city transportation) you incur as a result of participating in this study.

Privacy and Confidentiality

Information that you provide to us will be kept private. Only the research team at Dalhousie University will have access to this information. We will describe and share our findings in a class presentation and thesis while also vying for publishing in an academic journal. We will be very careful to only talk about group results so that no one will be identified. This means that

you will not be identified in any way in our reports. The people who work with your information have special training and have an obligation to keep all research information private. Also, we will use a participant number (not your name) in our written and computerized records so that the information we have about you contains no names. All of your identifying information will be kept in a separate file, in a locked cabinet, in a locked room. All electronic records will be kept secure in a password-protected, encrypted file on the researcher's personal computer (or on a Dalhousie University secure server) and all written documents will be stored in a locked cabinet where only the supervising professor will have access.

If You Decide to Stop Participating

You are free to leave the study at any time. If you decide to stop participating at any point in the study, you can also decide whether or not you want any of the information that you have contributed up to that point to be removed. You can also decide to have your information removed up to one month after the final testing.

How to Obtain Results?

You can obtain either group results or your individual results by including your contact information at the end of the signature page and we will send them to you via your preferred method.

Questions

We are happy to talk with you about any questions or concerns you may have about your participation in this research study. Please contact Susan Robinson at susan.robinson@dal.ca or Dr. Derek Kimmerly at dskimmerly@dal.ca or (902) 494-2570, at any time. We will also tell you if any new information comes up that could affect your decision to participate.

Catherine Connors, Director, Research Ethics, Dalhousie University at (902) 494-1462, or email: ethics@dal.ca

CONSENT FOR STUDY PARTICIPATION

Project Title: Can short-term ingestion of virgin coconut oil improve vascular function and exercise-mediated hyperemia in healthy young adults?

I, _____ have read the explanation about this study. I have been given the opportunity to discuss it and my questions have been answered to my satisfaction. I agree to take part in this study. However, I realize that my participation is voluntary and that I am free to withdraw from the study while it is ongoing, and up to one month following testing.

_____ _____

Participant's Signature *DATE*

_____ _____

Print Name of Participant *DATE*

_____ _____

Signature of Witness *DATE*

I confirm that I have explained the nature and purpose of the study to the participant names above and have answered all questions. In my judgment the participant is voluntarily and knowingly giving informed consent.

_____ _____ _____

Name of Person Obtaining Consent | Signature | Relationship to Participant

Please contact me at (please list a phone number, e-mail address, or mailing address):

_____

_____

_____

Please send me (please circle):

GROUP RESULTS **INDIVIDUALS RESULTS** **BOTH**

Appendix C: Health History Questionnaire

NAME:

DATE OF BIRTH:

EMAIL ADDRESS:

PARTICIPANT I.D. (Completed by Research Team)

Instructions to calculate your body mass index (BMI). If your BMI is greater than 30 kg/m^2 you will not be eligible to participate in the study.

1. What is your approximate weight (kilograms)? _____
   To convert from pounds to kilograms, multiply by 0.454

2. What is your approximate height (meters)? _____
   To convert from inches to meters, multiple by 0.0254

3. Please calculate your approximate BMI:

BMI (kg/m^2) = $\frac{\text{kg}}{(\text{m})*(\text{m})}$ = _____ = _____

These questions are designed to determine your eligibility for the study. If you answer 'Yes" to any question you will not be able to participate in the study.

1.	Have you been told to <u>not</u> engage in strenuous physical activity by a physician?	Yes	No
2.	Do you have diabetes?	Yes	No
3.	Do you have any bleeding or clotting problems?	Yes	No
4.	Have you ever had kidney disease?	Yes	No
5.	Have you ever had liver disease such as hepatitis?	Yes	No
6.	Have you ever had any thyroid disease?	Yes	No
7.	Have you smoked or consumed any nicotine containing product within the past 6 months?	Yes	No
8.	In the past 6 months, have you taken any medication that might stimulate or depress your nervous system (e.g. anti-depressants, amphetamines, Ritalin, Valium)?	Yes	No

For women only:

9.	Is there a possibility that you may be pregnant and/or are you breastfeeding?	Yes	No
10.	Do you have a regular menstrual cycle? (i.e., menstruating/ placebo pills of oral contraception during 7 days of a 28-day cycle)	Yes	No

Appendix D: Physical Activity Readiness Questionnaire

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2. Do you feel pain in your chest when you do physical activity?
☐	☐	3. In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
☐	☐	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7. Do you know of any other reason why you should not do physical activity?

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

continued on other side...

Appendix E: Daily Food Journal

Please indicate approximate portion sizes. Use back of sheet if needed. Please include drinks.

	Breakfast	Lunch	Dinner	Snacks
Monday	*Ex: 2 pieces of toast, 1 cup milk*	*Ex: sandwich (2 pc. bread, 2 slices ham, 2 pc lettuce, 1 slice cheese)*	*Ex: 1 chicken breast, 1 cup white rice, 1 cup mixed veg., 1 glass red wine.*	*Ex: 1 granola bar*
Tuesday				
Wednesday				
Thursday				
Friday				
Saturday				
Sunday				

Appendix F: Examples of VCO Recipes

*PLEASE **DO NOT** COOK VIRGIN COCONUT OIL*

Breakfast Ideas

Grain-Free No-Bake Protein Bars (8 tablespoons VCO in this recipe)

Ingredients

- 2 cups nuts or seeds (dried)
- ½ cup flax meal
- ½ cup seed or nut butter
- 3/8 teaspoon salt
- **½ cup virgin coconut oil**
- 2 tbsp liquid sweetener
- 2 tsp vanilla extract
- 1 cup chocolate chips

Instructions

- Place nuts or seeds, flax meal, coconut, seed or nut butter and salt in the bowl of a food processor.
- Process until the nuts or seeds are ground into a coarse meal
- Add room temp virgin coconut oil, sweeteners and vanilla to processor bowl and process until well combined to form a thick, yet crunchy paste.
- Press the mixture into an 8x8 square pan (you can be quite flexible here. A 9x9 will work just fine. A larger pan will produce thin bars, while a smaller pan will yield thicker ones.)
- Place in refrigerator to chill.
- While bars are chilling, melt chocolate chips.
- Top bars with melted chocolate
- Place back in refrigerator to chill
- Cut into squares and serve. (1/8 = 1 tbsp VCO)

Fruit and Nut Granola with Chia Seeds and Virgin Coconut Oil (8 tablespoons VCO in this recipe)

Ingredients (can substitute any

- 4 cups old fashioned oats
- ¼ cup dried cranberries
- ¼ cup dried cherries
- ¼ cup raisins
- ¼ cup walnuts
- ¼ cup almonds (sliced or not)
- ¼ cup sunflower seeds
- ¼ cup pumpkin seeds

- ¼ cup chia seeds
- **½ cup virgin coconut oil**
- ¼ cup honey
- ¼ tsp vanilla
- ½ tbsp. cinnamon.

Instructions

- Preheat oven to 250 degrees
- Melt honey and vanilla together and mix
- Mix nuts and seeds in large bowl
- Sprinkle cinnamon over dry mixture and stir
- Mix in honey/vanilla mixture
- Toast nut mixture in the oven or a pan until crispy
- Take off heat and mix in fruit and virgin coconut oil (do not cook in this study)
- Store in fridge to keep fresh

Chocolate Banana Shake with Virgin Coconut Oil (1 tablespoon VCO in this recipe)

Add to blender:

- 1 cup milk
- 1 tablespoon maple syrup
- 1 large ripe banana
- **1 tbsp virgin coconut oil (lightly melted)**
- 1 teaspoon vanilla extract
- 1 tablespoon cocoa powder

Add ice to thicken

Dinner Ideas

- Add VCO to pre-cooked stir-fry
- Mix VCO in a pre-heated soup
- Mix VCO into a curry sauce

Snack Ideas

- Lightly melt VCO and use on popcorn instead of butter

Appendix G: VCO Supplement Checklist

Check box when you have eaten tablespoon VCO.
One tablespoon at breakfast, one tablespoon at dinner.
Two tablespoons to be eaten per day.

	Week 1		Week 2		Week 3		Week 4	
	Breakfast	Dinner	Breakfast	Dinner	Breakfast	Dinner	Breakfast	Dinner
MON								
TUES								
WED								
THURS								
FRI								
SAT								
SUN								

Appendix H: Day-to-Day Variability of the FMD Test

Data from 10 participants (6 ♂, 23 ± 1 years, 27 ± 6 $kg \cdot m^{-2}$) were included in this analysis. The Bland-Altman scatterplot displays a mean difference of -0.069 ± 0.47, and a 95% limit of agreement between the two days ranging from -0.99 – 0.85 (Figure H). After testing the mean difference against a value of 0, it was determined that no fixed bias exists (p = 0.66). Proportional bias was also determined to be non-existent as linear regression analysis revealed that the slope of the regression line was not significantly different from zero (p = 0.42).

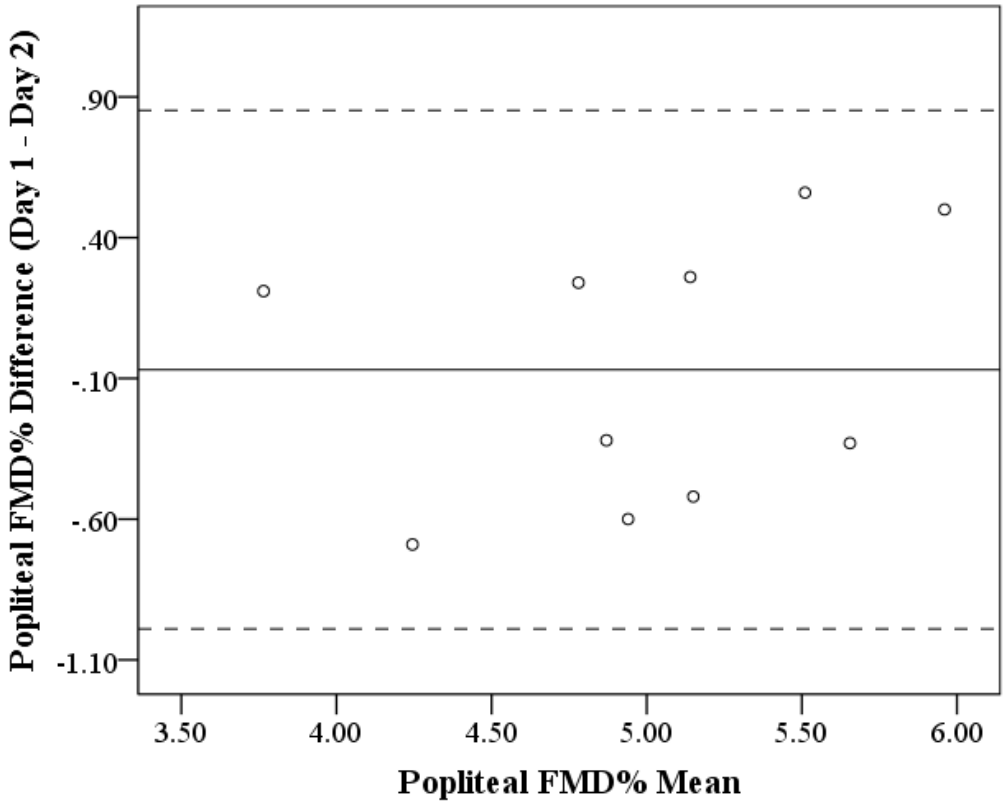

Figure H: Bland-Altman scatterplot illustrating close agreement in day-to-day popliteal artery FMD%, analyzed by the principal researcher. The middle solid line represents the mean difference of day-to-day popliteal FMD% (-0.069 ± 0.47) and the outer dotted lines represent the 95% limits of agreement (-0.99 to 0.85). Mean ± SDs of FMD% on Day 1 and Day 2 were 5.0 ± 0.8 % and 5.0 ± 0.6 %, respectively. FMD%, relative flow-mediated dilation.

Appendix I: TAC ELISA Assay Standard Curve

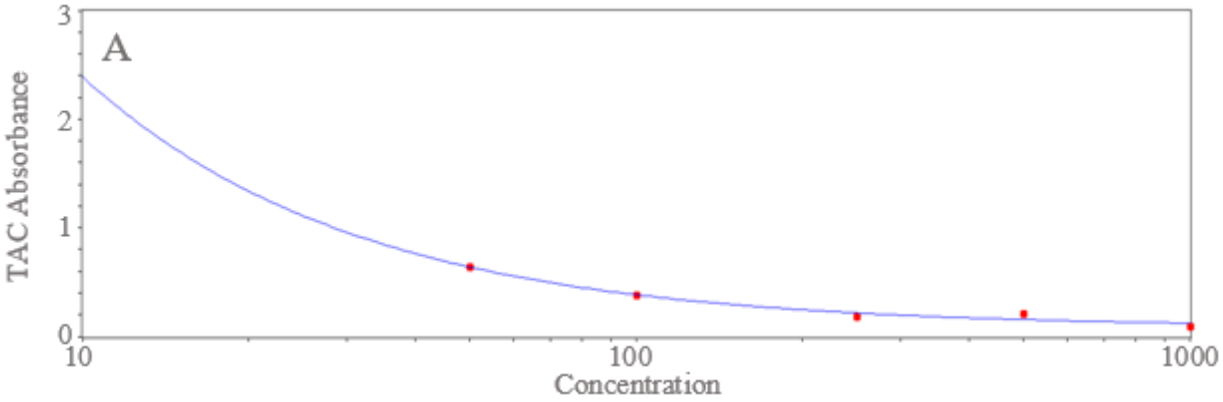

Figure Description: A) TAC standard curve ($ng \cdot ml^{-1}$); 5-parameter logistic regression equation: $y = ((0.085 \pm 0.015) - (93.947 \pm 27286433.613)) / ((1 + (X / (0.183 \pm 54535.833))^{(-0.897 \pm 1.320)})^{(0.911 \pm 507754.850)}) + (93.947 \pm 27286433.613)$; $R^2 = 0.972$. TAC, total antioxidant content.