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The Role of Cellular Aging in the Regulation of Free Fatty Acid Induced Cellular Inflammation by Liver X Receptor Alpha

Charles S. Schwartz
Virginia Commonwealth University

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

by

Charles Scott Schwartz
B.S. Virginia Commonwealth University 2015

Director: Edmund O. Acevedo, Interim Associate Dean for Finance and Administration, College of Humanities and Sciences

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Abstract

THE ROLE OF CELLULAR AGING IN THE REGULATION OF FREE FATTY ACID INDUCED CELLULAR INFLAMMATION BY LIVER X RECEPTOR ALPHA

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Purpose: This study examined the impact of an acute bout of exercise on LXRα mRNA expression in isolated PBMCs from aerobically fit middle-aged males, and its associations with cellular inflammation. Furthermore, association between cellular age and LXRα were investigated. **Methods:** LXRα mRNA, plasma free fatty acids, IL-6, and MCP-1 responses were quantified following acute moderate intensity exercise and throughout recovery. To examine LXRα's associations with cellular inflammation, PBMCs were stimulated with palmitate. Finally, telomere length was quantified as an indicator of cellular age. **Results:** LXRα mRNA expression was reduced at 90 minutes of recovery and did not influence systemic inflammation. Palmitate induced increases in LXRα were negatively associated with IL-6 and MCP-1. Furthermore, cellular age was associated with pre exercise palmitate induced LXRα. **Conclusion:** Palmitate induced LXRα response demonstrated the role of physical activity and cellular aging on the ability of LXRα to regulate cellular inflammation in immune cells.

Chapter 1: Introduction

It is estimated that over 40% of the United States population will be diagnosed with cardiovascular disease (CVD) by 2030, accounting for a $1.1 *trillion* financial burden to the healthcare system.[1] The pathophysiology of CVD is largely attributed to chronic inflammation within the vasculature.[2] Aging, an inevitable process that increases the risk for CVD, is accompanied by the chronic elevation of low-grade, systemic inflammation known as *inflamm-aging* that typically manifests between the ages of 40-64.[3,4] Specifically, elevations of systemic inflammation develop, in part, through the accumulation of centrally located visceral adipose tissue (VAT) that can occur without a change in body weight.[5,6] Therefore, the identification of mechanistic targets related to adiposity and aging that may slow and potentially reverse age related CVD pathology is of paramount importance.

VAT possesses a low capacity to store fatty acids (FA), eliciting an increase in systemic saturated fatty acids (SFA), such as palmitate,[5] that interact with toll-like receptor 4 (TLR4) on monocytes.[7,8] Palmitate ligation to TLR4 on monocytes initiates an intracellular signaling pathway that triggers the nuclear translocation of the nuclear factor-κB (NF-κB) transcription factor, and consequently, the production of pro-inflammatory cytokines (i.e., interleukin-6 [IL-6]) and chemokines (i.e., monocyte chemoattractant protein-1 [MCP-1]).[7,8] Furthermore, chronic exposure to the age-related pro-inflammatory milieu predisposes circulating monocytes to a pro-inflammatory phenotype referred to as M1, and to the increased infiltration and differentiation of monocytes to resident macrophages inside VAT, [9,10] thereby enhancing the *inflamm-aging* phenotype that is directly involved in the development and pathology of age-related CVD.[11–13]

Furthermore, cellular age can enhance the cellular shift towards proinflammatory subsets. Leukocyte telomere length is a cellular marker of biological age, and may be a more accurate

indicator of disease risk than chronological age.[14] Telomeres are gene poor regions located at the ends of chromosomes, which protect genomic DNA from damage during cellular replication.[15] Although telomere lengths shorten naturally due to cellular replication, the accumulation of stressors over time enhance telomere attrition and accelerate age-related ailments.[16] More worrisome, once telomeres reach critical length, the cell may enter into an irreversible state of cell cycle arrest known as senescence.[17] Cellular senescence promotes a pro-inflammatory environment, referred to as a senescence-associated secretory phenotype (SASP), which is driven by an overactivation of NFkB, following exposure to a pathogen, further contributing to *inflamm-aging*.[16,17]

A key protein along a mechanistic pathway to inflammation is liver x receptor α (LXRα), a nuclear transcription factor found in monocytes and monocyte-derived macrophages, in addition to other metabolic tissues.[18] While LXRα mRNA expression is elevated in M1 macrophages during CVD pathology,[19,20] animal studies have shown that the genetic elimination of the LXRα gene accelerates cellular apoptotic death, impairs basic immune function, advances the progression of atherosclerosis, and decreases survival from bacterial infection.[21,22] However, the reintroduction of LXRα from wild-type, genetically unmodified mice partially reverses this phenotype, supporting a cardioprotective role for LXRα.[21] More specifically, elevated cellular mRNA expression of LXRα facilitates cholesterol efflux and prevents the excess accumulation of lipids via mechanistic regulation of cellular cholesterol transporters such as ATP binding cassette 1 (ABCA1), serving as a mechanism to reduce foam cell formation and cellular apoptosis during the formation of atherosclerotic plaques.[23,24] In addition, LXRα is attributed to the inhibition of sequence-specific DNA binding by NF-κB following cellular exposure to the TLR4 ligand lipopolysaccharide (LPS),[25] thereby reducing the immune cell production of pro-

inflammatory cytokines and chemokines, including IL-6 and MCP-1, respectively.[26,27] These findings suggest that increased expression of LXRα decreases systemic inflammation, and support the need to identify therapeutic strategies that could possibly increase LXRα to prevent the progression of CVD.

Regular participation in moderate to vigorous physical activity is an anti-inflammatory behavior that exerts numerous metabolic benefits, and protects against CVD.[28] Specifically, aerobic exercise training increases fatty acid oxidation, and helps reverse the *inflamm-aging* phenotype by reducing adipocyte size, inhibiting monocyte infiltration into VAT, and inducing a switch of resident macrophages from an inflammatory M1, to an anti-inflammatory M2, phenotype. [28–30] Collectively, these beneficial adaptations also decrease circulating concentrations of palmitate that may help maintain the appropriate immune function of circulating monocytes.[28] Recently, it has been shown that 8 weeks of aerobic exercise training at a low to moderate intensity in young and middle-aged adults increases LXRα mRNA in circulating monocytes.[31,32] In addition, cycling at 70% of VO_{2max} for 45 minutes has been shown to increase LXRα mRNA expression in circulating peripheral blood mononuclear cells (PBMCs) following 90 minutes of recovery.[33] Furthermore, the mechanism for increased activity of LXRα has been supported by increased ABCA1 mRNA expression at 3 hours of recovery[33], and may also result in a reduction in pro-inflammatory cytokine production by monocytes, although no studies have been conducted to demonstrate this response.

Recent evidence suggests that there are tissue dependent alterations in LXRα mRNA expression throughout aging in animal models.[34–36] Although changes with aging have not been investigated in humans or in PBMCs, previous studies in animal models suggest a blunted LXR response in older individuals. Furthermore, in the presence of an LXRα ligand, cells with lower

expression of LXRα have reduced counter regulatory effects in response to an inflammatory stimulus.[27] Therefore, it could be deduced that fit older individuals would have greater LXRα activity in response to an acute bout of exercise in comparison to unfit individuals, due to increased baseline mRNA expression of LXRα, which would lead to an attenuation of pro-inflammatory cytokine production. This preliminary investigation of the underlying mechanisms involved with the regulation of inflammation by LXRα will focus on older fit individuals, who are susceptible to low-grade inflammation but should have an elevated LXRα response to acute exercise.

More specifically, the purpose of this study is to investigate the effects of an acute bout of moderate-intensity exercise on LXRα mRNA expression from isolated PBMCs, and its association with systemic FFA and the pro-inflammatory proteins IL-6 and MCP-1 in aerobically fit middle-aged males. Furthermore, to determine the capacity of LXRα to regulate inflammation following exercise, isolated PBMCs will undergo a 4-hour *ex vivo* cell stimulation with palmitate before and after an acute bout of exercise. It is hypothesized that LXRα mRNA expression at 90 minutes into recovery will be increased, compared to baseline. In addition, increased mRNA expression of LXRα will be associated with an attenuated *in vivo* and *ex vivo* pro-inflammatory milieu in trained middle-aged males. Finally, cellular age will predict LXRα mRNA expression and the pro-inflammatory response.

Specific Aims:

1. To investigate the relationship of LXRα to cellular inflammation in PBMCs in response to an acute bout of moderate intensity exercise in middle-age fit males.

   Hypothesis- Compared to pre-exercise, LXRα mRNA expression will be increased following 90 minutes of recovery. Increased expression of LXRα will be associated with decreased systemic concentrations of IL-6, MCP-1, and FFA.

2. To determine if LXRα mRNA expression is associated with palmitate induced production of IL-6 and MCP-1 from PBMCs isolated from middle-age fit males following acute moderate intensity exercise.

   Hypothesis- Increased expression of LXRα mRNA in PBMCs following exercise will be associated with attenuated production of IL-6 and MCP-1 following *ex vivo* palmitate stimulation. Furthermore, conflicting results in the literature limit the ability to make a directional hypothesis with regards to palmitate induced alterations in LXRα.

3. To determine if chronological age, in months, or cellular age, as assessed by telomere length, predicts LXRα's response to acute moderate intensity exercise in PBMCs.

   Hypothesis- Cellular age, but not chronological age will be associated with LXRα. More specifically, shorter telomere length will be associated with reduced LXRα mRNA expression at baseline, and in response to exercise, and increased levels of systemic IL-6, MCP-1 and FFA. Additionally, shorter telomere length will be associated with greater *ex vivo* production of IL-6 and MCP-1 in response to palmitate stimulation (see Figure 1 A and B).

Chapter 2: Literature Review

1. Aging and *Inflamm-aging*

Aging is an inevitable process that is linked to a myriad of diseases that are attributed to an increase in chronic, systemic, low-grade inflammation, known as *inflamm-aging.*[4] *Inflamm-aging* is the culmination of many inflammatory processes including altered energy metabolism, cellular senescence, and the overactivation of the pro-inflammatory arm of the innate immune system.[4] Adipose tissue is a major source of inflammation during aging, and in many ways, the excess secretion of pro-inflammatory proteins derived from morphological changes of adipocytes influences immune cell function, and contributes to a feed-forward mechanism that exacerbates the pro-inflammatory state of the *inflamm-aging* phenotype.[37] Therefore, determination of potential strategies to disrupt the feed-forward mechanism associated with adipose tissue inflammation is of tremendous importance.

1.1 Adipocyte Morphology

Adipose tissue plays an integral role in the storage and release of FFA in response to metabolic demand.[38] In addition to storing FFA, adipose tissue is a large endocrine organ that releases hormones and adipokines to influence appetite, control energy balance, and regulate immune function.[38,39] During the aging process there is a shift from peripherally located adipose tissue, to centrally located adipose tissue.[5] Centrally located adipose tissue consists of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). SAT is located below the skin, and is responsible for storage of lipids,[40] whereas VAT is located within the abdominal cavity, providing support for the visceral organs, and has a dense capillary network.[40] When SAT adipocytes hypertrophy, the capacity of SAT to expand and store lipids becomes limited. As a

result, VAT begins to accumulate, serving as an additional storage site for lipids.[40] This accumulation and subsequent expansion of VAT is linked to a chronic, systemic pro-inflammatory milieu, and is a potent risk factor for CVD and other metabolic diseases.[41] In addition, hypertrophied VAT releases pro-inflammatory cytokines, such as IL-6 and TNFα, and chemokines, such as MCP-1.[41] This pro-inflammatory signaling promotes the infiltration of adipose tissue by monocytes, which differentiate into resident adipose tissue macrophages (ATM).[41] Additionally, VAT has a lower storage capacity for FFA when compared to SAT, allowing for FFA to spill over into circulation.[5]

1.2 Immune Cell Distribution in Adipose Tissue

Adipose tissues can be further broken down into adipocytes and the stromal vascular fraction (SVF).[11,13,41] The SVF consists of monocyte derived macrophages, and adipose tissue stromal cells.[13] During the aging process, there is an increased hypertrophy in VAT, stimulating a pro-inflammatory milieu.[11,13] This is a result of macrophage phenotype within the SVF.[13] ATM differentiate into one of two major phenotypes: the classically activated pro-inflammatory, M1, macrophage and the alternatively activated anti-inflammatory, M2, macrophage.[42] More specifically, M1 macrophages release pro-inflammatory cytokines (i.e., IL-1β, IL-6, and TNF-α) and chemokines (i.e., MCP-1) that contribute to systemic inflammation, and further supporting a feed-forward mechanism by promoting monocyte infiltration and their subsequent differentiation into M1 macrophages. To the contrary, M2 macrophages release anti-inflammatory cytokines (i.e., IL-10 and tumor growth factor beta), thereby promoting the resolution of tissue inflammation.[28] Recent studies have shown that total macrophages residing in aged adipose tissue does not differ from young adipose tissue,[11,13] yet ATM are more likely to shift towards an M1 macrophage phenotype.[13] Likewise, aged adipocytes have increased mRNA expression of

pro-inflammatory cytokines when compared to young adipocytes.[11] While the extent to which the increased gene expression of these pro-inflammatory cytokines contribute to systemic inflammatory profiles is unknown,[13] the resulting pro-inflammatory milieu associated with *inflamm-aging* predisposes circulating monocytes towards a pro-inflammatory macrophage phenotype upon their entry into tissues.

1.3 Circulating Monocytes and Subset Shift

Monocytes are a heterogeneous population of leukocytes that play an important role in modulating innate immunity and serve as the first line of host defense in response to pathogens.[9,43] In humans, circulating monocytes are categorized as *classical* ($CD14^{++}CD16^{-}$), *non-classical* ($CD14^{dim}CD16^{+}$), and *intermediate* ($CD14^{++}CD16^{+}$).[42] Immune cells, such as monocytes and monocyte derived macrophages express pattern-recognition receptors (PRRs) that detect pathogen-associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs). TLR4 is a well characterized PRRs that preferentially binds to the endotoxin lipopolysaccharide (LPS) in a manner dependent upon CD14 and myeloid differentiation factor 2 (MD2) surface expression. Upon ligation, this CD14-MD2-TLR4 complex activates myeloid differentiation factor 88 (MyD88) to initiate an intracellular signaling cascade that promotes the translocation of the nuclear factor (NF)-κB transcription factor into the cell's nucleus. NF-κB is the primary transcription factor that stimulates the gene expression and release of the pro-inflammatory cytokines IL-1β, IL-6 and TNFα.[8,44] Interestingly, monocyte populations expressing CD16 are considered pro-inflammatory, expressing higher levels of TLR4 that are responsible for the increased production of pro-inflammatory cytokines compared to the *classical* monocyte subset. Furthermore, aging is accompanied by the increased proportion of *non-classical*, $CD14^{low}CD16^{+}$, monocytes that may contribute to the *inflamm-aging* phenotype.[45]

Circulating SFA also activate TLR4 on monocytes, promoting the production of pro-inflammatory cytokines.[7] For example, a recent study by Pararasa and colleagues investigated the effects of aging on fatty acid profiles, and monocyte polarization.[5] During healthy aging there is an increase in circulating concentrations of FFA, including total SFA.[5] Briefly, FA were measured in plasma from young adults (24.16 ± 3.76 years) and middle age adults (57.53 ± 6.07 years). There were significant increases in C14, and C24 SFA's, whereas C16, the most prevalent SFA in circulation, was unaltered.[5] Middle aged adults also had significantly higher concentrations of the pro-inflammatory cytokines IL-6 and TNFα, and significantly lower concentrations of the anti-inflammatory cytokine IL-10.[5] Additional *in vitro* investigations using THP-1 cells, a model cell line that mimics the functions of monocytes, have shown that SFA, regardless of chain length, primed monocytes towards an M1, pro-inflammatory phenotype, while unsaturated FA primed monocytes towards an M2, anti-inflammatory phenotype.[5] These results suggest that the spillover of SFA from VAT and the increase pro-inflammatory profiles of circulating monocytes exacerbate the progression of pro-inflammatory disease pathology, including CVD.

2.4 Cardiovascular Disease

Atherosclerosis is amongst the leading pathological contributors to CVD complications,[46] and is characterized by a pro-inflammatory milieu and an imbalance in cellular lipid metabolism that contribute to the accumulation of lipid laden macrophages within the arterial intima.[47] During the initial stages of atherosclerosis, low density lipoprotein (LDL) particles become trapped in the arterial intima, and are modified by oxidative stress or enzymatic cleavage.[24] Modified LDL particles, such as oxidized LDL (oxLDL), promote a pro-inflammatory environment that activate the vascular endothelium and increase the production and release of

chemoattractant proteins (chemokines).[24] As a result, the release of chemokines creates a gradient that stimulates classical and intermediate monocytes to migrate towards the vascular endothelium, transmigrate into the endothelial space, and ultimately, differentiate into M1, pro-inflammatory macrophages.[24]

Resident M1 macrophages ingest the LDL particles through phagocytosis, and the uptake oxLDL via scavenger receptors contributes to the excess buildup of cholesterol within the macrophages and the formation of foam cells.[24] Foam cells promote an atherosclerotic environment by enhancing the release of pro-inflammatory cytokines and chemokines, further influencing monocyte transmigration as a feed-forward loop.[24] Improper cholesterol handling within foam cells may induce excess endoplasmic reticulum (ER) stress that results in cellular death by apoptosis and the formation of a necrotic core that is a hallmark characteristic of atherosclerotic lesions.[24] The necrotic core continues to increase in size due to smooth muscle cell proliferation and apoptosis.[24] As the necrotic core increases in size, it has the potential to decrease blood flow to downstream tissues, and more worrisome, the rupture of the necrotic core could potentially cause the complete occlusion of blood flow and tissue necrosis.[24] Therefore it is important to investigate mechanistic targets, such as LXRs, that slow and potentially reverse the progression of atherosclerosis.

2. Liver X Receptors

Liver X Receptors (LXRs) are a family of ligand activated nuclear transcription factors that regulate cholesterol trafficking and the intracellular inflammatory signaling pathway within immune cells.[48] LXRs are naturally activated by oxysterols and other byproducts of cellular cholesterol metabolism.[48] There are two isoforms of LXRs; LXRα (NR1H3) and LXRβ (NR1H2).[48] LXRα is found in most metabolic tissues, including the liver, kidneys, and

monocytes/macrophages, while LXRβ is present in nearly every tissue.[48] Both LXRα and LXRβ have important roles in cholesterol efflux from macrophages by stimulating ATP binding cassette (ABC) A1, and ABCG1.[48] The ability to influence cholesterol balance and inflammation highlighting the need to further study LXRs as a therapeutic target to protect against CVD.

3.1 LXR and Cardiovascular Disease

LXRα, and to a lesser extent LXRβ, have been shown to protect against the formation of atherosclerotic plaques in mice.[21] Briefly, mice lacking LXRα had increased atherosclerotic lesion area when compared to mice expressing LXRα.[21] However, atherosclerotic lesion size was reversed upon transplantation of bone marrow from mice expressing LXRα in LXRα null mice.[21] Likewise, studies investigating the impact of LXR signaling have demonstrated their direct atheroprotective effects. More specifically, synthetic LXR agonists, such as TO-901317 and GW3965, have been used in many *in vitro* and *in vivo* models to activate LXR.[49] Although these LXR agonists increase fatty acid production in mice, resulting in hypertriglyceridemia and the formation of fatty liver diseases,[49] *in vitro* models support the efficacy of LXRs for regulating inflammation.[26,27,50] For example, LXRnull mice injected with LPS present with increased circulating IL-6 compared to wild type controls.[27] At the cellular level, pretreating the RAW 264.7 macrophage cell line for 18 hours with LXR agonist, prior to infection with *Escherichia coli* or LPS stimulation, inhibits the production of inducible nitric oxide synthase (iNOS), and inflammatory markers cyclooxygenase-2, IL-1β, IL-6, and MCP-1.[27] However, the inhibition of these inflammatory markers was reduced in LXRα and LXRβ knockout macrophages, and nonexistent in LXRnull macrophages.[27] The potential anti-inflammatory effects of LXR ligands have been corroborated in human studies. For example, human mononuclear cells pretreated with LXR ligands for 0 or 6 hours prior to LPS stimulation secreted lower levels of MCP-1 and TNFα

compared to LPS stimulation alone.[51] Likewise, similar studies in THP-1 derived macrophages have shown that pretreatment with an LXR agonist reduced mRNA expression of the inflammatory markers IL-1β, IL-6, and TNF-α in THP-1 derived macrophages.[50] Pretreatment of adherent human monocytes with differing concentrations of an LXR agonist reduced inflammatory proteins including IL-1β, IL-6, IL-8, IL-10, TNF-α, IL-12p40, macrophage inflammatory protein (MIP) 1α, MIP-β, and MCP-1 in the cell supernatant, in a dose-dependent manner.[26] MCP-1 and IL-10 were only influenced at the highest concentration of the LXR agonist.[26] mRNA expression of IL-6, IL-8 and TNF-α were not influenced by LXR activation.[26] These results from *in-vivo* and *in-vitro* investigations highlight the novel role of LXRs as a regulator of innate immune function, and further indicate that an exogenous ligand to promote LXR signaling could prove to be beneficial for reducing and potentially reversing the accumulation of atherosclerotic plaques.

Further studies indicate that LXRα is more important for regulating inflammation.[22,26] LXRα is the key isoform that protects bone marrow-derived and peritoneal macrophages from cellular apoptosis following exposure to *listeria monocytogenes* infection.[22] Joseph et al. demonstrated that mice lacking LXRα (LXRα$^{-/-}$ and LXRαβ $^{-/-}$) were more susceptible to *listeria monocytogenes*-induced cellular death by apoptosis compared to wild type, or mice that lack LXRβ (LXRβ$^{-/-}$).[22] Furthermore, LXRα mRNA expression in human monocytes is upregulated following LPS stimulation, whereas LXRβ was not influenced.[26] Indicating that LXRα, but not LXRβ, plays a role in the innate immune response to an endotoxin.[26] These results suggest that LXRα is an important mediator of inflammation during the innate immune response,[22,26] and warrants additional research focusing on understanding the role of LXRα as a mechanistic target to reduce the inflammatory burden associated with the aging process.

3. Physical Activity

Regular participation in aerobic physical activity is an anti-inflammatory behavior and is known to protect against CVD and other metabolic disorders.[28] Many of the anti-inflammatory benefits can be attributed to exercise induced alterations to adipose tissue.[28] For example, exercise training reduces VAT area, without changing body weight in obese humans.[52] Consequently, plasma FFA concentrations are also reduced following exercise training,[52] suggesting that physical activity aids in the regulation of lipid metabolism, or reduction of excess storage of fatty acids in adipose tissue.[53] During aerobic exercise, trained males have increased mobilization, uptake, and oxidation of FFA when compared to their untrained counterparts working at the same relative workload.[54] Furthermore aerobic exercise has been shown to reduce mRNA expression of pro-inflammatory proteins (e.g. RANTES, CCR5, TNF-α, and IL-6) in SAT from obese humans,[55] inhibit the infiltration of monocytes into VAT, and elicit a phenotypical switch from M1 to M2 macrophages.[30] These results suggest that aerobic exercise training could aid in reducing the chronic systemic low-grade pro-inflammatory milieu.

Similar benefits have been observed following acute exercise, evidenced by reductions in IL-1β, TNF-α, and MCP-1 mRNA, and increased IL-10 mRNA in SVF and adipocytes in obese rats.[29] Acute exercise has also been shown to elicit the increased expression of the M2 marker, macrophage galactose-type lectin-1 (MGL1), indicating a shift towards M2 macrophages.[29] While these results show the efficacy of exercise to help reduce the *inflamm-aging* phenotype, less is known about whether or not aerobic exercise supports the appropriate immune function by positively influencing LXR in circulating monocytes.

4.1 Chronic Aerobic Exercise and LXR mRNA Expression

In rodents, chronic endurance training increases LXRα mRNA expression in a variety of tissues, including the liver and skeletal muscle.[56–58] Endurance training increases LXRα mRNA expression in the liver samples harvested from male Wistar rats.[56,57] Furthermore, these adaptations occur in as little as 4 weeks.[57] Conversely, adaptations to LXRα mRNA expression were not seen in hepatocytes harvested from female Sprague-Dawley rats following 8 weeks of endurance training, suggesting a gender specific adaptation to aerobic exercise training.[59] In skeletal muscle, LXRα regulates the storage and oxidation of intramuscular triglycerides.[60] More specifically, LXRα directly influences fatty acid synthase in rats overexpressing peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α; a signaling protein known to increase with exercise),[58] supporting the role of LXR as a regulator of lipid metabolism in skeletal muscle, and may be a contributing factor to increased intramuscular triglyceride stores following exercise training.

In humans, chronic aerobic exercise has been shown to increase LXRα mRNA expression in PBMCs following low and moderate intensity exercise.[31,32] In a study by Butcher et al.,[32] thirty-four healthy, sedentary adults (18 males, 16 females; aged 45 ± 11.1 years old) completed 10,000 steps on a treadmill at a self-selected pace, 3 times per week, for 8 weeks, resulting in the increased expression of LXRα mRNA in PBMCs.[32] These changes were also linked to positive adaptations of cholesterol metabolism, evidenced by significant reductions in total cholesterol and increased concentration in HDL cholesterol.[32] Additionally, Ruffino et al.[31] showed significant increases in LXRα mRNA following 8 weeks of walking (55 - 69% of APHRM) for 45 minutes, 3 times a week in nineteen sedentary adult females (aged 42 ± 11 years). Furthermore, gene expression of inflammatory markers in monocytes demonstrated a priming for

M2 differentiation, evidenced by significant increases in IL-10 and dectin-1 (M2 markers), and decreases in MCP-1 (M1 marker).[31] These changes may also have positive effects on cholesterol trafficking as demonstrated by a reduction in triglycerides following the intervention.[31]

These results support the efficacy of regularly participating in moderate intensity aerobic exercise as a cardioprotective habit. Furthermore, there were positive effects on blood lipid profiles following training, which may be resultant of LXRα's influence on cellular cholesterol metabolism. Reduction of M1 primed monocytes may also reduce the formation of foam cells, further reducing the risk of developing atherosclerotic plaques. However, no studies have investigated the LXRα's ability to regulate inflammation in response to aerobic exercise training. Such studies would provide additional mechanistic insight into potential regulatory mechanisms to support the anti-inflammatory effects of chronic aerobic exercise training that contribute to the reduced risk of CVD.

4.2 Acute Aerobic Exercise and LXR mRNA Expression

Little is known about the impact of acute aerobic exercise on LXR expression and functional capacity. For example, a recent study by Davies & colleagues [61] demonstrated that acute exercise on a cycle ergometer (70% VO_{2max}) for 45 minutes elicited a non-significant increase ($p < 0.10$) in LXRα mRNA expression at 3 hours post exercise in five physically active adult males.[61] On the other hand, Thomas et al.[33] observed significant increases in LXRα mRNA levels at 1.5 hours post exercise (45 minutes of aerobic exercise on a cycle ergometer at 70% $VO_{2max)}$ in nine healthy active, but not endurance trained, participants (32 ± 8 years old) following participation.[33] Interestingly, these increases were not sustained at 3 hours post exercise, suggesting that either aerobic training status influences LXRα mRNA expression patterns following acute aerobic exercise or LXRα acts transiently during the initial phases of

recovery. Therefore, more evidence is necessary in determining whether acute exercise increases LXRα. Further investigation would help determine if LXRα potentiates the inflammatory response in PBMCs following acute exercise. Results from such studies would provide additional support for acute exercise as an effective LXRα agonist, providing a mechanistic understanding for this nonpharmacological approach to protect against CVD.

4. Conclusion

LXRα is a transcription factor which plays an important role in the progression of atherosclerosis via the regulation of pro-inflammatory signaling in circulating monocytes. Increased circulating SFA is associated with age related changes in VAT, and further contributes to *inflamm-aging* via TLR4 ligation. Exercise training is known to increase LXRα mRNA expression in PBMC's, but this has not been associated with the regulation of pro-inflammatory proteins linked to age-related increases in circulating concentrations of SFA (Specific Aim 1). Likewise, while acute exercise has been shown to increase LXRα mRNA expression in PBMC's, no studies have investigated how LXR expression relates to systemic inflammation in trained middle-aged adults in response to acute exercise (Specific Aim 1). Furthermore, no studies have investigated LXRα's ability to regulate palmitate-induced inflammation in PBMC's in an *ex vivo* model (Specific Aim 2). Finally, no studies have investigated the influence of age, cellular or chronological, on LXRα's response to acute moderate intensity exercise (Specific Aim 3). Results from such study could provide further understanding of the role of LXRα in innate immunity, and its ability to potentially disrupt the feed forward inflammatory mechanisms associated with *inflamm-aging*.

Chapter 3: Methodology

Subject Population

Aerobically fit, middle-aged male runners between the ages of 40-65 were recruited for this study. Prior to all laboratory activities participants provided informed consent and completed a brief medical history questionnaire, and the International Physical Activity Questionnaire short form (IPAQ-SF)[62]. The IPAQ-SF was used to quantify participation in physical activity and categorize subjects as participating in high physical activity. Specifically, individuals who were categorized as participating in high levels of physical activity by the IPAQ-SF accumulated ≥ 3000 MET-minutes · wk^{-1} of walking, moderate activity or vigorous activity, over 7 or more days, or accumulating ≥ 1500 MET-minutes · wk^{-1} of vigorous intensity physical activity over 3 or more days.[63] Furthermore, potential subjects were excluded from participation if they possessed a body mass index classification (BMI) ≥ 30 kg/m^2, have any known or suspected cardiovascular, metabolic, rheumatologic, or other inflammatory diseases/conditions, or a history of cancer within the last ten years. Additionally, subjects taking medication known to alter the immune system or metabolism, users of tobacco products, or consumed greater than an average of ten alcoholic beverages per week were excluded from participation.

Laboratory Procedures

Participants arrived for both laboratory sessions between 6:30 and 7:30 AM, following an overnight fast for a minimum of 8 hours. In addition, subjects were instructed to abstain from caffeine, alcohol, painkilling medications, and intense exercise for at least 24hr. Upon arrival for their first visit, subject's height and weight were recorded, and body mass index was calculated. In addition, waist and hip circumference was assessed using a Gulick tape and waist-to-hip ratio

was calculated, and visceral adiposity was measured across the sagittal plane at the horizontal levels of the L4 and L5 vertebrae.[64] Finally, total body fat percentage was assessed using air displacement plethysmography with measured thoracic gas volume (Bod Pod; Cosmed, Concord, CA., USA).

Exercise Testing Procedures

Session two took place a minimum of 72 hours following session one. Upon arrival, participants were fitted with a heart rate monitor (Polar Electro Oy, Kempele, Finland), and seated for a 15-minute rest period to determine resting heart rate and blood pressure. Participants then completed a 10-minute warmup, allowing for the prediction of maximal oxygen consumption (protocol listed below). Immediately following the warmup, participants completed 30 minutes of treadmill exercise at 60% of their predicted VO_{2max}. Exercise intensity was monitored using real-time oxygen consumption (VO_2) values via open circuit spirometry (ParvoMedics, Sandy, UT) and was adjusted every 5 minutes to ensure a consistent workload.

VO_{2max} was predicted utilizing the protocol presented by Vehrs and colleagues.[65] Briefly, subjects began with a short warm-up stage consisting of 5 minutes of walking at a self-selected pace, at 0% grade. Following the warm-up stage, the treadmill speed was increased to a self-selected jogging pace between 4.3 and 7.5 mph. Subjects jogged at this pace for 3 minutes or until steady state HR is reached. Steady state HR was defined as a difference of ≤ 3 bpm, between two consecutive HRs over 30 seconds. Steady state HR and treadmill speed was input into the following equation for the calculation of VO_{2max}: VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$) = 58.687 + (7.520 x Gender; 0 = female and 1 = male) + (4.334 x mph) – (0.211 x kg) – (0.148 x HR) – (0.107 x age. This prediction equation has been validated in individuals between the ages of 18

and 40 (R = 0.91, SEE = 2.52 mL · kg^{-1} · min^{-1})[65] and has previously been used to estimate VO_{2max} in men over 60 years.[66]

Blood Collection and Preparation

Whole blood samples were obtained from the subject's antecubital vein using a 22G intravenous catheter (BD Nexiva™ closed IV catheter system, Sandy, UT) and collected into tubes containing K2 ethylenediaminetetraacetic acid (K2EDTA; BD Vacutainer, Franklin Lakes, NJ) prior to (PRE), immediately upon completion of exercise (POST), and 30 (R30) and 60 (R60) and 90 (R90) minutes into recovery following exercise. Whole blood samples were centrifuged at 3000rpm for 20 minutes for plasma isolation. Plasma was stored at -80ºC in cryogenic tubes for the analysis of IL-6 and MCP-1 using enzyme linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA) techniques, and FFA using a commercial colorimetric assay (MAK044; Sigma-Aldrich, St. Louis, MO, USA).

Following plasma aspiration, the leukocyte buffy coat was isolated and transferred to a conical tube, then diluted in saline to a final volume of 5mL. The white buffy coat suspension was then layered onto an equal amount of Histo-Paque (p=1.077 g/mL; Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 400g for 30 minutes. PBMCs were then collected and washed three times with saline. Isolated PBMCs were homogenized in TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) for long-term storage at -20°C or resuspended in 1mL RPMI 1640 media (Invitrogen, Carlsbad, CA, USA), manually counted by hemocytometer, and adjusted to a final volume of $2.0x10^6$ cells/mL for cell culture (described below).

Preparation of Palmitate Solution for Cell Culture

Palmitate solution was prepared as previously described.[67–69] Briefly, sodium palmitate (Sigma Aldrich, St. Louis, MO, USA) was dissolved in 0.01 M sodium hydroxide (NaOH; Fisher Scientific; Hampton, NH, USA), at a final concentration of 8 mmol/L and incubated at 70ºC for 30 minutes to ensure palmitate was fully dissolved. Palmitate was then conjugated with 1 mmol/L fatty acid free bovine serum albumin (BSA; Sigma Aldrich, St. Louis, MO, USA) in 1x phosphate buffered saline (PBS; Fisher Scientific; Hampton, NH, USA)) for 15 minutes at 50ºC at an 8:1 fatty acid to BSA molar ratio.

Cell Culture

PBMCs were cultured in RPMI 1640 media supplemented with 5% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 1% penicillin, and 1% streptomycin (Life Technologies, Carlsbad, CA, USA) in a 6-well culture plate (Corning Incorporated, Corning, NY, USA) at $2.0x10^6$ cells/mL. Plated cells were stimulated with 200 μmol/L of palmitate solution or left unstimulated and incubated at 37ºC at 5% CO2 for 4 hours. This concentration of palmitate has been previously shown to induce the production of pro-inflammatory cytokines in PBMCs.[67,70] Unstimulated cells served as a time-course control. Following the 4-hour culture period, the cell culture supernatant was aspirated and centrifuged at 2000 rpm for 5 minutes at 4°C, to pellet any suspended PBMCs. Cell culture supernatant was transferred to cryogenic tubes and stored at -80ºC for future analysis of IL-6 and MCP-1 using ELISA techniques (R&D Systems, Minneapolis, MN, USA). In addition, pelleted PBMCs were recombined with adherent cells, homogenized with TRIzol® reagent, and stored as previously described.

mRNA Isolation & Measurement

PBMCs (cultured and uncultured) homogenized in TRIzol® reagent were added to QIAshredder mini spin columns (Qiagen, Hilden, Germany) and centrifuged at 16,000g for 2 minutes to ensure complete homogenization. Total RNA was then isolated as per the TRIzol® reagent manufacturer instructions. Total RNA quantification and purity were assessed using the NanoDrop lite (Thermo Scientific, Waltham, MA, USA). 1000ng of total RNA was reverse transcribed to complementary DNA (cDNA) using qScript cDNA synthesis kit (QuantaBio, Beverly, MA, USA) as according to manufacturer instructions. TaqMan probes (Applied Biosystems, Foster City, CA, USA) were used in conjunction with TaqMan gene expression master mix (Applied Biosystems, Foster City, CA, USA) for reverse transcription-polymerase chain reaction (RT-PCR) as according to manufacturer's instructions. LXRα mRNA (HS00172885_m1) measurement was conducted using RT-PCR methods, with eukaryotic 18S (HS99999901_s1) as a reference gene. PCR thermocycler (C1000 Touch; Bio-Rad, Hercules, CA, USA) conditions were 50ºC for 2 minutes, 95ºC for 10 minutes, followed by 40 cycles of denaturation at 95ºC for 15 seconds and annealing at 60º for 60 seconds. All RT-PCR samples were run in triplicate. Relative LXRα expression was calculated using $2^{-\Delta\Delta Ct}$.

Telomere Length Measurement

Telomere length was quantified using relative T/S ratio, as described by Cawthon.[71] Briefly, DNA was isolated from PRE PBMCs homogenized in TRIzol reagent® as according to manufacturer's instructions. DNA was quantified using the NanoDrop lite. PCR reactions were prepared using 2x SYBR green master mix (QuantaBio, Beverly, MA, USA), target primers (sequences as described by Cawthon[71]; Integrative DNA Technologies, Skokie IL, USA),

nuclease free water, and 15ng DNA. All PCR reactions were run in triplicate. PCR thermocycler (Applied Biosystems 7500; Foster City, CA, USA) conditions for telomeres were 95ºC for 2 minutes, followed by 40 cycles of denaturation at 95 ºC for 15 seconds and annealing at 54ºC for 2 minutes. PCR thermocycler conditions for 36B4 were 95 ºC for 2 minutes, followed by 40 cycles of denaturation at 95ºC for 15 seconds, and annealing at 58 ºC for 1 minute.

Statistical Analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 25.0). Data are presented as mean ± standard error of the mean (SEM) unless otherwise stated. Statistical significance was set as $p \leq 0.05$.

Aim 1: Changes in PBMC mRNA expression of LXRα and systemic concentrations of FFA and the pro-inflammatory proteins IL-6 and MCP-1 prior to and in response to exercise were analyzed using repeated measures analysis of variance (rmANOVA). Post-hoc analyses were conducted to determine differences between time points. Associations between LXRα mRNA expression and plasma SFA, IL-6, and MCP-1 concentrations were examined using Pearson's correlation.

Aim 2: Palmitate induced changes in LXRα were analyzed using paired t-tests. Furthermore, changes in LXRα due to exercise and palmitate were assessed using a two-way rmANOVA. Post-hoc analyses were conducted to determine differences between time points. Associations between palmitate induced cytokine production and LXRα expression were assessed using Pearson's correlation.

Aim 3: Associations between chronological age, and cellular age and LXRα, and cytokine responses were analyzed using Pearson's correlation.

Chapter 4: Results

Subject Characteristics

Fourteen apparently healthy, aerobically fit, middle-aged runners (age 50 ± 8.28 years) participated in this study. All subjects participated in high levels of physical activity in accordance with IPAQ-SF (3600 ± 1282.34 MET-mins · week^{-1}). Furthermore, subjects met BMI criteria for the study (24.26 ± 2.0 kg · m^{-2}). Subject characteristics can be found in Table 1. Subject characteristics are displayed as mean ± sd.

Exercise Session

All subjects completed the described exercise protocol. The average heart rate and treadmill speed to predict VO_{2max} (50.97 ± 2.79 mL · kg^{-1} · min^{-1}) were 134.07 ± 10.11 bpm and 6.07 ± 0.49 mph, respectively. Furthermore, the workload completed (30.67 ± 1.76 mL · kg^{-1} · min^{-1}) was 0.42 ± 1.57% of the target workload (30.56 ± 1.67 mL · kg^{-1} · min^{-1}). Average exercise session variables are listed in Table 2. Moreover, 5-minute averages of exercise variables are listed in Table 3. All exercise session variables are displayed as mean ± sd.

Exercise induced alterations of LXRα mRNA expression in circulating PBMCs, and associations with systemic markers of *inflammation*

LXRα mRNA expression in circulating PBMCs was significantly decreased at 90 minutes into recovery (R90) compared to PRE (p = 0.037) and POST (p = 0.016) (Figure 2A). Furthermore, plasma FFA was significantly altered (increase then decrease) in response to exercise and throughout recovery ($F_{[2,12]}$ = 7.167, p = 0.009) (Figure 2B). In addition, Plasma IL-6 was significantly increased following exercise and throughout recovery ($F_{[4,12]}$ = 4.507, p = 0.007) (Figure 3A). Likewise, plasma MCP-1 was significantly altered (increase then decrease)

following exercise and throughout recovery ($F_{[4,10]} = 3.988$, $p = 0.035$) (Figure 3B). LXRα mRNA at R90 was negatively associated with plasma IL-6 concentrations at POST (r = -0.584, p = 0.028) and R30 (r = -0.579, $p = 0.030$). In addition, this relationship approached significance at R60 (r = -0.530, $p = 0.051$), and R90 (r = -.450, $p = 0.106$) (Table 4). Furthermore, there were no associations between LXRα mRNA expression in circulating PBMCs and plasma FFA, nor plasma MCP-1 at any time point.

Palmitate induced alterations of LXRα mRNA expression in *ex vivo* stimulated PBMCs, and associations with IL-6 and MCP-1

Ex vivo palmitate stimulation increased LXRα mRNA expression in PBMCs when compared to unstimulated conditions at every time point ($p \leq 0.05$) (Figure 4A). Additionally, there was a significant interaction between time and palmitate stimulation when compared to unstimulated PRE cells ($F_{[1.776,23.093]} = 4.497$; $p = 0.026$) (Figure 4B). Moreover, LXRα mRNA exhibited elevations that were approaching significance in unstimulated POST cells ($p = 0.074$) and were significantly increased in unstimulated R90 cells ($p = 0.020$) when compared to unstimulated PRE cells, demonstrating an exercise effect on LXRα (filled bars in Figure 4B). Furthermore, PBMC's collected at R90 exhibited an exacerbated response (R90palm difference from unstimulated R90) following exposure to palmitate compared to POST (POSTpalm difference from unstimulated POST; p = 0.007), which was approaching significance PRE (PREpalm difference from unstimulated PRE; p = 0.076) demonstrating an additive effect of exercise and palmitate (Figure 4B).

As expected, palmitate also increased IL-6 production in PBMCs collected at PRE (p = 0.00), POST ($p = 0.009$), R30 ($p = 0.009$), R60 ($p = 0.005$), and R90 ($p = 0.008$) when compared to unstimulated PBMCs (Figure 5A). Likewise, palmitate significantly increased MCP-1

production in PBMCs collected at PRE (p = 0.007), POST (p = 0.013), R30 (p = 0.012), and R60 (p = 0.020) and near significant at R90 (p = 0.052) when compared to unstimulated PBMCs (Figure 5B). However, the exercise conditions did not demonstrate an altered response in IL-6 and MCP-1.

Palmitate induced LXRα mRNA expression in PRE PBMCs were negatively correlated with IL-6 production in response to palmitate (IL-6 difference) at PRE (-0.623; p = 0.017), POST (-0.539; p = 0.047), R30 (-0.535; p = 0.048), and R60 (-0.549; p = 0.042), and this relationship approached significance at R90 (-0.515; p = 0.060) (Table 5A). Likewise, palmitate induced LXRα mRNA expression in POST PBMCs was negatively correlated with the IL-6 difference at POST (r = -0.654; p = 0.011), R30 (r = -0.0650; p = 0.012), R60 (r = -0.614; p = 0.019), and R90 (r = -0.629; p = 0.016) (Table 5A). Palmitate induced LXRα mRNA expression in R90 cells were not significantly associated with IL-6 production. Furthermore, palmitate induced LXRα mRNA expression in POST PBMCs was negatively correlated with palmitate induced changes in MCP-1 (MCP-1 difference) at R90 (r = -0.537; p = 0.048) (Table 5B).

Relationship between Chronological age, Cellular age and LXRα

No relationship existed between LXRα mRNA expression in circulating PBMCs and chronological age, in months, at any time point. Additionally, chronological age was not associated with the plasma FFA, plasma IL-6, or plasma MCP-1. Likewise, there were no relationships between chronological age and palmitate-induced LXRα or cytokine production (Table 6). Cellular age, as assessed by telomere length (T/S ratio), was positively associated with palmitate induced LXRα in PRE cells (r = 0.569; p = 0.034), however this relationship was not present in POST or R90 cells (Table 6). Moreover, telomere length was not associated with LXRα expression in circulating PBMCs, or plasma FFA, plasma IL-6 or plasma MCP-1.

Furthermore, there was no relationship between telomere length and palmitate induced cytokine production.

Chapter 5: Discussion

This study sought to investigate the effects of an acute bout of moderate-intensity exercise on LXRα mRNA expression, and its association with systemic markers of inflammation in trained middle-aged males. Observations from this study show LXRα mRNA expression in circulating PBMCs was not increased 90 minutes into recovery as hypothesized, but instead, was decreased. Furthermore, a negative relationship existed between LXRα mRNA expression in circulating PBMCs collected at 90 minutes of recovery and plasma IL-6, but not plasma MCP-1 or plasma free fatty acids. *Ex vivo* experiments exhibited increased LXRα mRNA expression following palmitate stimulation immediately before, after and at 90 minutes of recovery, and these increases were negatively associated with palmitate induced increases in IL-6 and MCP-1. This is the first study to demonstrate the relationship of LXRα to cellular inflammation in response to palmitate following an acute bout of exercise.

Currently, the impact of an acute bout of exercise on LXRα mRNA in circulating PBMCs remains unclear. For example, LXRα mRNA expression has been shown to be significantly increased at 90 minutes into recovery following 45 minutes of cycling at 70% of VO_{2max}, however returned to pre exercise levels at 3 hours of recovery.[33] Conversely, the same exercise protocol elicited elevations in LXRα mRNA expression that approached significance at 3 hours of recovery, and was not reported at 90 minutes of recovery.[61] Finally, the present data shows a reduction in LXRα mRNA expression in circulating PBMCs at 90 minutes into recovery after 30 minutes of treadmill exercise at 60% of predicted VO_{2max}. It is unknown what factors may influence LXRα following acute exercise, however it is possible that the acute bout of exercise used in the present study may not have been potent enough to elicit increased expression of LXRα mRNA in trained middle-aged individuals. Interestingly, when compared to unstimulated

PBMCs collected immediately before exercise, LXRα mRNA expression was increased in unstimulated PBMCs collected at 90 minutes of recovery, following 4 hours of *ex vivo* conditions. These results suggest that LXRα increases in response to exercise, although the response in circulating PBMCS may extend past the hypothesized 90 minutes of recovery, or may be influenced by an unknown LXRα mediator in circulation. Furthermore, the cellular mechanisms regulating LXRα in circulating PBMCs following acute exercise, in parallel with the immunoregulatory and metabolic pathways in monocytes and PBMCs, remain obscure.

Results from the current study show plasma IL-6 increasing in response to an acute bout of exercise, and remaining elevated at 90 minutes of recovery. These results are consistent with those previously presented in a young, physically active population where 40 minutes of exercise at 55 or 65% of VO_{2max} elicited significant increases in systemic IL-6 concentrations, and continued to increase until the end of exercise at 60 minutes.[72] In addition, elevated systemic concentrations of IL-6 persisted until 3 hours of recovery, although it is unclear if the final 20 minutes of exercise may have influenced IL-6 during recovery.[72] Additionally, previous literature investigating untrained middle aged adults show no changes in serum IL-6, following 30 minutes of walking at 50% of VO_{2max}.[73] These results suggest that training may preserve the IL-6 response to acute exercise in middle aged individuals.

Plasma MCP-1 was significantly increased at 30 minutes of recovery, then returned to pre exercise levels at 90 minutes of recovery. A number of studies indicate that changes in circulating MCP-1 following acute exercise may be dependent on fitness status, body composition, and exercise stimuli.[74–76] For example, circulating MCP-1 was significantly increased in trained runners following 1 hour of treadmill running at 60% of VO_{2max}, and remained elevated 1 hour following exercise.[74] Furthermore, this response was exacerbated

following 45 minutes of downhill running at 60% of VO_{2max}.[74] Additionally, 20 minutes of treadmill exercise at 70% of VO_{2max} elicited decreases in circulating MCP-1 in sedentary normal weight individuals, whereas it was unchanged in those categorized as overweight-moderately obese and severely obese.[75] Moreover, circulating MCP-1 remained unchanged in both overweight and obese and lean untrained individuals following a bout of cycling at 55-60% of their max heart rate for 120 minutes.[76] The MCP-1 response in trained middle aged subjects in this study is similar to the response of the other trained groups supporting a training effect for MCP-1.

Results from the present study demonstrate a negative association between relative LXRα expression in circulating PBMCs at 90 minutes of recovery, and plasma IL-6 concentrations immediately following exercise, and throughout recovery. Although these association exists, it is unknown if LXRα in circulating monocytes mediates the plasma IL-6 response following exercise. Furthermore, there is evidence that monocytes do not significantly contribute to changes in systemic IL-6 in response to exercise.[77,78] Conversely, IL-6 has been shown to exert anti-inflammatory effects in response to an acute inflammatory stimulus. For example, exercise induced IL-6 or recumbent IL-6 infusion elicited a reduction in pro-inflammatory cytokine production following intravenous endotoxin infusion.[79] Thus, it is possible that exercise induced increases in IL-6, likely released from the muscle, may attenuate downstream LXRα expression in trained middle-aged individuals, however this response has not been elucidated in the literature.

LXRα's pivotal role in the regulation of pro-inflammatory cytokine production in monocytes and macrophages has been well documented. In a murine RAW 264.7 macrophage cell line, cells pretreated with an LXR agonist, prior to infection with *Escherichia coli*, or LPS

stimulation, exhibited lesser production of many pro-inflammatory makers, including IL-6 and MCP-1.[27] Furthermore, this response was attenuated in peritoneal macrophages harvested from LXRα knockout, and LXRnull mice.[27] These potential anti-inflammatory effects of LXR ligands are supported in human studies. For example, in human mononuclear cells pretreated with LXR ligands for 0 or 6 hours prior to LPS stimulation produced lower concentrations of MCP-1 and TNFα, compared to LPS alone.[51] Likewise, pretreatment with an LXR agonist reduced the mRNA expression of a number of pro-inflammatory markers, including IL-6, in THP-1 derived macrophages, and exposed to LPS.[50] Additionally, when adherent human monocytes were pretreated with differing concentrations of an LXR agonist, inflammatory protein production was reduced in a dose dependent manner following exposure to LPS.[26] In corroboration with previous findings, the results from the current study show that LXRα has a negative relationship with pro-inflammatory cytokine production in response an inflammatory stimulus, such as palmitate. Moreover, this response was influenced by an acute bout of exercise evidenced by greater negative association immediately following exercise, and lack of associations at 90 minutes of recovery.

Observed increases in LXRα mRNA expression following *ex vivo* palmitate stimulation in human PBMCs have not been shown previously, and differ from those presented in a murine cell line. Few studies have examined the influences of TLR-4 ligation on LXRα mRNA expression. Previous investigations have stimulated murine RAW264.7 cells with differing concentrations of palmitate (400 μM and 100 μM), and for a longer duration (16-20 hours, respectively) than the current study, and showed no LXRα response.[80,81] Additionally, human PBMCs stimulated with LPS for 6 hours show increased expression of LXRα mRNA.[26] Conflicting results suggest that LXRα's response to TLR-4 ligation may be due to differences in

cell type, however no studies have been done to elucidate this response. Specifically, previous literature has shown no correlation in the production of IL-6 between RAW264.7 macrophages and human PBMCs when comparing a variety of immunomodulators in response to LPS or *E. coli*.[82]

Additionally, increased LXRα mRNA expression at R90 were exacerbated following palmitate stimulation, indicating that the LXRα response to palmitate is potentiated by exercise. The mechanisms responsible for this exacerbated response are unclear. However, it is possible that the exercise effect on LXRα occurred via intracellular mechanism such as peroxisome proliferator activated receptor gamma coactivator 1-alpha (PGC1α), which has been shown to be elevated following acute exercise and may be linked to increased LXRα activity in monocytes.[33,61] In addition to the exercise effect, palmitate ligation to TLR4 likely further increased LXRα mRNA expression.[26] It is unclear if this potentiated response may influence cellular inflammation in trained middle-age individuals.

As expected, chronological age was not associated with LXRα mRNA expression in circulating PBMCs, or plasma FFA, IL-6 or MCP-1, nor palmitate induced changes in LXRα mRNA expression, or IL-6 and MCP-1 production. Interestingly, cellular age was positively associated with palmitate induced increases in LXRα in cells collected immediately before but not post exercise, or 90 minutes into recovery. These results indicate that the LXRα response may be preserved in those with longer telomeres in response to TLR4, and this response is altered with exercise. The relationship between telomere length and LXRα is unknown, however there is evidence that inhibition of LXRα mRNA increases human telomerase reverse transcriptase (hTERT) which is responsible for maintaining telomere length.[83] hTERT has been shown to be upregulated by NF-κB activation, which may explain increased hTERT expression

after LXRα inhibition.[84] While this is the case, there may be a reduced need for hTERT in PBMCs from trained individuals, as telomere length may be preserved due to the anti-inflammatory milieu resultant of chronic exercise. Furthermore, previous investigations in our lab have shown reduced hTERT expression following LPS stimulation in PBMCs from older individuals, who presented with shorter telomeres, however inflammatory cytokine production was unaltered, suggesting that shorter telomere length in circulating PBMCs may not influence inflammation.[6] It may also be the case that the plethora of intracellular reactions that occur during exercise influence the interaction between telomeres length and LXRα. Further research is necessary to determine the influence of telomere length on cellular inflammation, and cell function.

Future investigations should consider the following limiting factors when interpreting results from this investigation. Due to the omission of a comparison group, this investigation was unable to determine if age or fitness levels may influence the LXRα at baseline or in response to either exercise or palmitate, and how these responses are related to cellular age. In addition, this investigation did not utilize LXRα agonists or inhibitors, which would further elucidate the mechanisms involved with LXRα's regulation of inflammation following exercise. Furthermore, this investigation only focused on the regulation of the pro-inflammatory cytokines in the inflammatory response, while LXRα may also regulate the anti-inflammatory cytokines.

In conclusion, this investigation demonstrated that LXRα mRNA expression is altered in PBMCs following an acute bout of exercise in trained middle-aged males, albeit the mechanisms driving these alterations remain obscure. Additionally, results from the e*x vivo* experiments further support LXRα as a regulator of inflammation in PBMCs. Interestingly, exercise potentiated LXRα mRNA expression following palmitate stimulation. These observations are of

particular interest to older unfit individuals who present with increased visceral adiposity, increased resident M1 macrophages, and increased levels of circulating SFA. Specifically, participation in an acute bout of exercise which complies to public health recommendations may stimulate increases in LXRα activity in circulating monocytes, possibly extending to resident macrophages, and reduce systemic levels of pro-inflammatory cytokines. Furthermore, this study supports exercise as a stimulator of LXRα, and may be an alternative to pharmacological interventions which may have negative health implications.

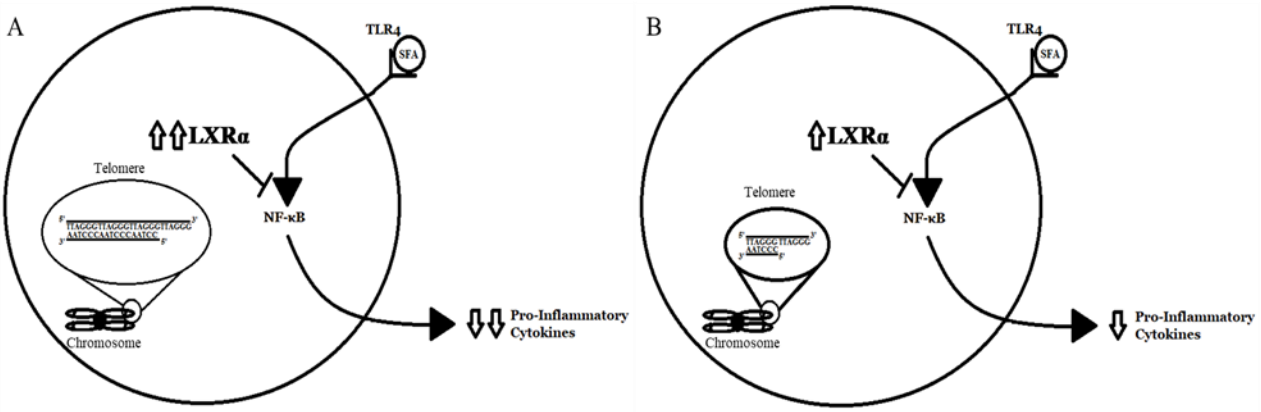

Figure 1. Proposed relationship between LXRα and telomere length. Longer telomere length will be associated with a greater increase in LXRα in response to acute exercise, and greater attenuation of palmitate induced pro-inflammatory cytokine production (panel A). Likewise, shorter telomere length will be associated with lesser increases in LXRα in response to acute exercise, and lesser attenuation of palmitate induced pro-inflammatory cytokine production (panel B).

Table 1. Subject Characteristics (n = 14)

Characteristic	Mean ± SD	Range
Age (years)	50.64 ± 8.28	41.0 - 65.0
Age (months)	614.29 ± 98.39	498.36 - 789.96
Height (cm)	178.34 ± 6.25	167.3 - 188.0
Weight (kg)	77.16 ± 7.67	64.3 - 87.9
BMI ($kg \cdot m^{-2}$)	24.26 ± 2.0	21.0 - 28.4
Saggital Diameter (cm)	23.5 ± 1.86	21.0 - 28.0
Body Fat (%BF)	19.0 ± 5.61	10.0 - 26.9
Waist (cm)	85.21 ± 4.86	77.0 -93.0
Hip (cm)	96.82 ± 3.78	90.0 - 101.5
Waist : Hip Ratio	0.88 ± 0.04	0.8 - 0.95
IPAQ (MET-mins · $week^{-1}$)	3600 ± 1282.34	1653.0 - 5892.0
Resting HR (bpm)	57.5 ± 3.08	54.0 - 62.0
Resting Systolic BP (mmHg)	114 ± 6.47	104.0 - 130.0
Resting Diastolic BP (mmHg)	79.43 ± 6.63	64.0 - 90.0
Predicted VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	50.97 ± 2.79	44.9 - 55.9
Realtive Telomere Length (T/S ratio)	1.02 ± 0.24	0.74 - 1.46

Note: Data are presented as mean ± SD. BMI: body mass index; IPAQ: international physical activity questionnaire; HR: heart rate; BP: blood pressure; VO_{2max}: maximal oxygen uptake.

Table 2. Exercise Session Variables (n = 14)

Variable	Mean ± SD	Range
Prediction HR (bpm)	134.07 ± 10.11	116.0 - 145.0
Prediction speed (mph)	6.07 ± 0.49	5.2 - 7.1
Target VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	30.56 ± 1.67	26.9 - 33.5
Exercise VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	30.69 ± 1.76	26.65 - 33.5
Percentage of Target VO_2	0.42 ± 1.57	-1.57 - 3.69
Exercise HR (bpm)	135.54 ± 10.48	113.3 - 149.3
Peak HR (bpm)	142.00 ± 10.86	117.0 - 154.0
Exercise RER ($VCO_2 \cdot VO_2^{-1}$)	0.86 ± 0.42	0.80 - 0.92
Kcal burned	343.74 ± 38.23	287.46 - 398.53

Note: Data are presented as mean ± SD. HR: heart rate; VO_2: oxygen consumption; RER: respiratory exchange ratio

Table 3. Exercise Session Variables: 5 Minute Averages

	Time (min)					
	Five	**Ten**	**Fifteen**	**Twenty**	**Twenty-five**	**Thirty**
Variable	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
HR (bpm)	131.07 ± 10.15	132.76 ± 10.21	134.4 ± 10.97	136.56 ± 11.04	138.71 ± 11.04	139.79 ± 11.33
RPE	10.36 ± 1.78	10.36 ± 1.95	10.29 ± 2.13	10.5 ± 2.07	10.57 ± 2.10	10.71 ± 2.13
Absolute VO_2 ($L \cdot min^{-1}$)	2.41 ± 0.27	2.35 ± 0.25	2.35 ± 0.25	2.35 ± 0.28	2.38 ± 0.27	2.36 ± 0.26
RelativeVO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$)	31.23 ± 1.48	30.51 ± 1.99	30.45 ± 1.86	30.46 ± 2.03	30.8 ± 1.85	30.65 ± 1.73
RER ($VCO_2 \cdot VO_2^{-1}$)	0.88 ± 0.05	0.86 ± 0.04	0.86 ± 0.04	0.85 ± 0.05	0.85 ± 0.05	0.84 ± 0.05

Note: Data are presented as mean ± SD. HR: heart rate; RPE: rating of perceived exertion, borg scale; VO_2: oxygen consumption; RER: respiratory exchange ratio

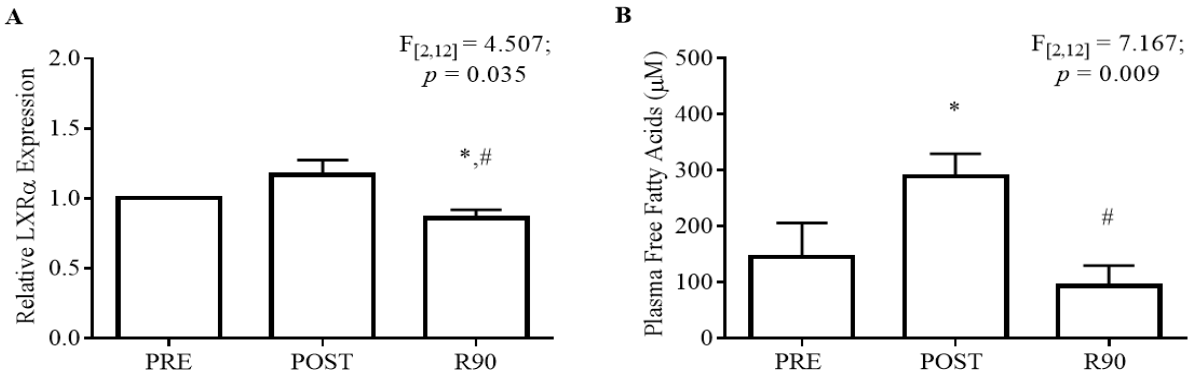

Figure 2. Exercise induced changes in LXRα from circulating PBMCs, and Plasma Free Fatty Acids. Relative LXRα expression was significantly reduced at 90 minutes of recovery (R90; panel A) Plasma free fatty acids were significantly increased immediately following exercise (POST), but returned back to baseline levels at 90 minutes of recovery (R90; panel B). * indicates significant change from PRE ($p \leq 0.05$). # indicates significant difference from POST ($p \leq 0.05$).

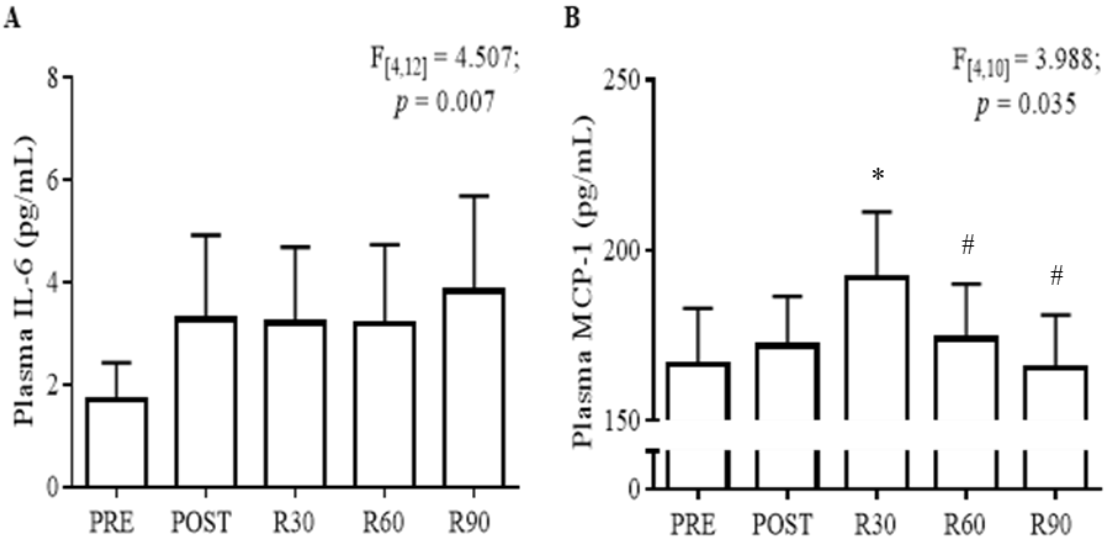

Figure 3. Plasma IL-6 and MCP-1 concentrations were significantly altered in response to exercise (panels A and B, respectively). * indicates significant difference from PRE ($p \leq 0.05$). # indicates significant difference from R30 ($p \leq 0.05$).

Table 4. Associations among relative LXRα expression and plasma IL-6 concentrations

	Plasma IL-6 pg/mL				
	PRE	POST	R30	R60	R90
POST LXRα	r = -0.415	r = -0.403	r = -0.398	r = -0.390	r = -0.346
	p = 0.14	p = 0.154	p = 0.159	p = 0.168	p = 0.225
R90 LXRα	r = -0.529	r = -0.562*	r = -0.557*	r = -0.533*	r = -0.488
	p = 0.052	p = 0.037	p = 0.039	p = 0.050	p = 0.077

* indicates a significant relationship between relative LXRα expression and plasma IL-6 concentrations ($p \leq 0.05$)

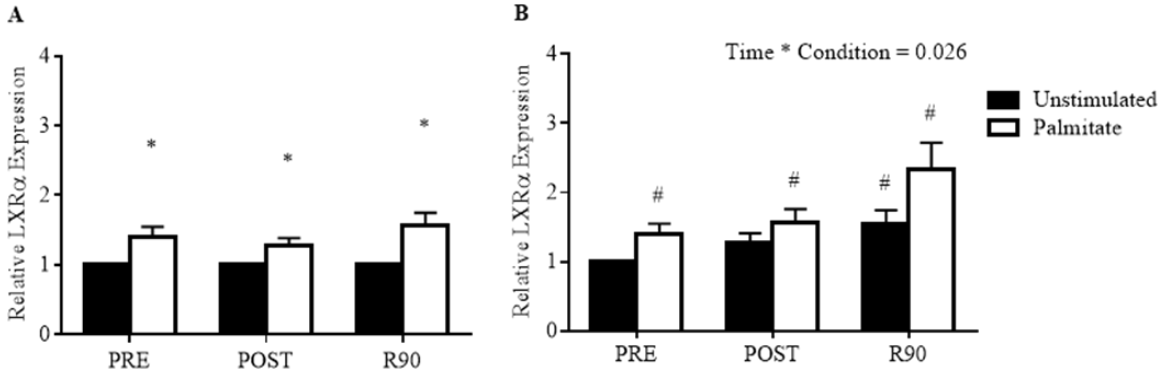

Figure 4. Relative LXRα expression changes following *ex vivo* palmitate stimulation. Palmitate induced changes in relative LXRα expression compared to unstimulated cells (panel A). Changes in relative LXR α expression due to exercise (filled bars), and exercise + palmitate (empty bars)(panel B). * indicates significant increases from unstimulated condition ($p \leq 0.05$). # indicates significant increases from unstimulated PRE condition ($p \leq 0.05$).

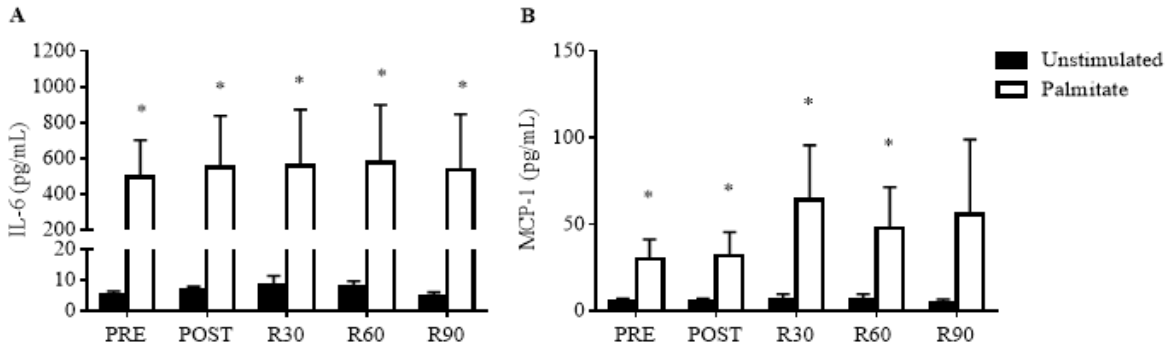

Figure 5. Cellular production of IL-6 and MCP-1 following palmitate stimulation. * indicates significant increase from unstimulated conditions ($p \leq 0.05$).

Table 5. Associations among palmitate induced LXRα and relative differences in IL-6 & MCP-1

	A. Relative difference in IL-6				
	PRE	POST	R30	R60	R90
PRE LXRα	r = -0.623* p = 0.017	r = -0.539* p = 0.047	r = -0.535* p = 0.048	r = -0.549* p = 0.042	r = -0.515 p = 0.060
POST LXRα		r = - 0.654* p = 0.011	r = -0.650* p = 0.012	r = -0.614* p = 0.019	r = -0.629* p = 0.016
R90 LXRα					r = -0.411 p = 0.145
	B. Relative difference in MCP-1				
	PRE	POST	R30	R60	R90
PRE LXRα	r = -0.183 p = 0.531	r = -0.178 p = 0.543	r = -0.332 p = 0.246	r = -0.325 p = 0.256	r = -0.375 p = 0.187
POST LXRα		r = -0.510 p = 0.062	r = -0.491 p = 0.075	r = 0.418 p = 0.137	r = 0.537* p = 0.048
R90 LXRα					r = -0.285 p = 0.324

* indicates a significant relationship between palmitate induced LXRα expression and relative differences in IL-6 & MCP-1 (A and B respectively; $p \leq 0.05$)

Table 6. Associations among palmiate induced LXRα expression, cellular age, and telomere length

	Palmitate Induced LXRα Expression		
	PRE	POST	R90
Chronological Age (Months)	r = 0.351 p = 0.219	r = 0.203 p = 0.486	r = -0.043 p = 0.885
Telomere Length (Relative T/S Ratio)	r = 0.569* p = 0.034	r = 0.009 p = 0.977	r = 0.087 p = 0.766

* indicates a significant relationship between palmitate induced LXRα expression and telomere length ($p \leq 0.05$)

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Curriculum Vita

Charles Scott Schwartz was born on October 3, 1990, in Fairfax County, Virginia. He attended William Monroe High School, graduating class of 2009, in Stanardsville, Virginia. He received his bachelor of sciences in Health, Physical Education, and Exercise Science with a concentration in Exercise Science at Virginia Commonwealth University in 2015.

EDUCATION

Master of Science: Health, Exercise and Movement Sciences
Virginia Commonwealth University, Richmond, VA
Concentration: Exercise Science
Faculty Advisor: Edmund O. Acevedo, Ph.D., FACSM, FAPA, FNAK
Anticipated Graduation: December 2019

Thesis (In Progress): *The role of Physical Activity in the Regulation of Free Fatty Acid Induced Cellular Inflammation by Liver X Receptor Alpha*

Bachelor of Science: Health, Physical Education, and Exercise Science
Virginia Commonwealth University, Richmond, VA
Concentration: Exercise Science
Graduated, August 2015

RELEVENT EXPERIENCE

VCU Department of Kinesiology and Health Sciences Graduate Teaching Assistant

Course Responsibilities:

- *†HPEX 353 Disease Trends, Prevention, and Control- Spring 2016, Fall 2016
- HPEX 435 Health Disparities in the United States- Spring 2016, Fall 2016
- HPEX 391 Exercise Psychology- Spring 2017
- HPEX 358 Intro to Epidemiology- Spring 2017
- HPEX 345 Nutrition for Health and Disease- Spring 2017
- HPEX 375 Exercise Physiology- Spring 2017
- HPEX 441 Assessment and Exercise Intervention- Fall 2017
- HPEX 470 Exercise Programming and Leadership- Fall 2017
- *HPEX 310 Fitness and Health- Fall 2017, Spring 2018, Fall 2018, Spring 2019
- *HPEX 375 Physiology of Exercise Lab- Spring 2018

* Indicates independent co-instructor position; † indicates online format

VCU Exercise Physiology Research Laboratory Assistant 2015 - 2017

- Responsible for general maintenance and upkeep of lab equipment including:
    - ParvoMedics True One 2400 Metabolic Cart
    - Sensormedics VMAX Metabolic Cart
    - Biohit mLine micropipettes

    - Cosmed Bod Pod
- Ensured proper training of laboratory staff on how to calibrate and collect data using laboratory equipment

VCU Exercise Physiology Research Laboratory Intern 2014 - 2015

- Conducted metabolic testing using Parvo Medics Metabolic Cart
    - Maximal & submaximal exercise testing
    - Basal metabolic rate
- Quantification of blood lactate levels using LactateScout+ during exercise
- Determination of Lactate Threshold
- Calculation of Ventilatory Threshold using WinBreak software
- Assistance in basic blood processing

VCU Men's Basketball Sports Science & Performance Intern 2014 - 2015

- Monitored practice heart rates in real time using Polar Team 2 software
- Produced daily training reports based on heart rate and metabolic data
- Produced weekly graphs based on training reports for coaching staff
- Weekly collection of saliva samples for analysis of hormones associated with overtraining syndrome

PROFESSIONAL MEMBERSHIP

- Southeast ACSM Member (2014 - Present)
- ACSM Member (2015 - Present)

CERTIFICATIONS

- ACSM Certified Exercise Physiologist (ACSM EP-C)

DEPARTMENT OR CAMPUS ACTIVITIES

- VCU Men's Basketball Liaison for Performance Testing, July 2017 - July 2018
- VCU Women's Basketball Liaison for Performance Testing, July 2017 - July 2018
- VCU Exercise Physiology Research Laboratory Journal Club - Spring 2015
- VCU Exercise Science Club, Vice President, Fall 2014 - Spring 2015
- VCU Exercise Science Club, Secretary, Spring 2014 - Fall 2014

RESEARCH EXPERIENCE

Accepted Peer Reviewed Publications

1. Slusher, A.L., Patterson, V.T., **Schwartz, C.S.**, & Acevedo, E.O. (2018). Impact of high intensity interval exercise on executive function and brain derived neurotrophic factor in healthy college aged males. *Physiology & behavior*, *191*, 116-122.

Manuscripts in Review

1. Morgan, J.A., Franco, R.L., Blanks A.M., Caslin, H.L., Patterson V.T., **Schwartz, C.S.,** Williams III, D.S.B.
   Effects of a 16-week training program on the relationship between leg stiffness and energy expenditure in middle-aged half-marathon runners

2. Patterson, V.T., Slusher, A.L., **Schwartz, C.S.** Acevedo, E.O.
Perceptual Responses and Physiological Activation with Acute High Intensity Interval Ergometry in Sedentary Males

Published Abstracts Presented at National Conferences

1. **Schwartz C.S.**, Slusher A.L., Zúñiga T.M., Shah K.K., Acevedo E.O. *Inflamm-aging* is Associated with Impairing the Process of Maintaining Telomere Length in LPS Stimulated PBMCS. *ACSM Annual Meeting.* Orlando, FL (May 2019)

2. **Schwartz C.S.**, Slusher A.L., Patterson V.T., Acevedo E.O. (2018) Impact of High-Intensity Interval Exercise on Executive Function and Brain Derived Neurotrophic Factor. *Medicine & Science in Sports & Exercise, 50(5S),* 94*. ACSM Annual Meeting.* Minneapolis, MN *(May 2018)*

3. Blanks, A.M., **Schwartz, C.S**., Pedersen, L.N., Buckley, L.F., Acevedo, E.O., Abbate, A., & Franco, R.L. (2017). Post-stemi Age-related Non-classical Monocyte Ccr2 Expression Differences In Response To Stress Hormones. *Medicine & Science in Sports & Exercise*, 49(5S), 1028. *ACSM Annual Meeting*. Denver, CO (May 2017)

4. Blanks, A.M., Bowen, M.K., Caslin, H.L., **Schwartz, C.S.**, Acevedo, E. O., & Franco, R.L. (2016). Exercise Recovery Index as an Assessment of Sympathetic Activity in Obese and Non-obese Males. *Medicine and science in sports and exercise*, *48*(5S), 1014. *ACSM Annual Meeting*. Boston, MA (May 2016)

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Regional Conference Presentations

1. **Schwartz C.S.**, Slusher A.L., Zúñiga T.M., Shah K.K., Acevedo E.O. *Inflamm-aging* is Associated with Impairing the Process of Maintaining Telomere Length in LPS Stimulated PBMCS. *Southeast Chapter ACSM Conference*. Greenville SC (February 2019)

2. Shah K.K., Slusher A.L., Zúñiga T.M., **Schwartz C.S**, Acevedo E.O. Excessive Weight Gain Throughout Adulthood is Associated with Shorter Telomere Lengths, Pro-inflammation, and Psychological Stress. *Southeast Chapter ACSM Conference*. Greenville SC (February 2019)

3. **Schwartz C.S.**, Slusher A.L., Patterson V.T., Acevedo E.O. Impact of High-Intensity Interval Exercise on Executive Function and Brain Derived Neurotrophic Factor. *Southeast Chapter ACSM Conference*. Chattanooga, TN (February 2018)

4. A.M. Blanks, M.K. Bowen, H.L. Caslin, **C.S. Schwartz**, E.O. Acevedo, R.L. Franco. Exercise Recovery Index as an Assessment of Sympathetic Activity in Obese and Non-Obese Males. *Southeast Chapter ACSM Conference*. Greenville, SC (February 2016)

5. Patterson V.T., Franco R.L., Morgan J., Caslin H.L., Crabb E.B., **Schwartz C.S.,** Williams D.S. Relationship of Adiposity and Running Kinematic Variables in Females. *Southeast Chapter ACSM Conference*. Jacksonville, FL (February 2015)

6. J.A. Morgan, **C.S. Schwartz**, V.T. Patterson, R.L. Franco, D.S. Williams. Lower Extremity Stiffness Influences Running Performance in Recreational Runners. *Southeast Chapter ACSM Conference*. Jacksonville, FL (February 2015)