Paraoxonase Responses to Exercise and Niacin Therapy in Men with Metabolic Syndrome

by

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ABSTRACT

Our purpose was to characterize changes in paraoxonase 1 (PON1) activity and concentration after single aerobic exercise sessions conducted before and after 6-weeks of niacin therapy in men with metabolic syndrome (MetS). Twelve men (44 ± 7 yrs; BMI = 34.5 ± 3.4 kg·m²; % fat = 35 ± 5; \( \dot{V}O_{2\text{max}} = 27.5 ± 6.6 \text{ mL min}^{-1} \text{kg}^{-1} \); waist circumference = 108.8 ± 8.2 cm; HDL-C = 39 ± 8 and triglycerides = 287 ± 96 mg·dL^{-1}; HOMA score = 4.4 ± 2.0) expended 500 kcals by treadmill walking at 65% of \( \dot{V}O_{2\text{max}} \) before and after a 6-week regimen of prescription niacin. Niacin doses were titrated by 500 mg·wk\(^{-1}\) from 500 to 1500 mg·dy\(^{-1}\) and maintained at 1500 mg·dy\(^{-1}\) for the last 4 weeks. Fasting blood samples were collected before and 24 hours after each exercise session and analyzed for PON1 activity, PON1 concentration, myeloperoxidase (MPO), apolipoprotein A1 (Apo-A1), and oxidized LDL (oLDL) concentrations and NMR analyses of lipoprotein characteristics. PON1 activity, PON1 concentration, MPO and oLDL were unaltered following the independent effects of acute exercise and six-weeks of niacin therapy. HDL particle size decreased by 3% (from 8.6 ± 0.1 to 8.5 ± 0.1 nm) following acute exercise (\( p = 0.040^* \)). Small LDL particle numbers were reduced by 16% (from 1480.0 ± 111.2 to 1244.6 ± 128.8 nmol·L\(^{-1}\)) following six weeks of niacin therapy (\( p = 0.057 \)). PON1 activity increased 6.1% (from 115.9 ± 24.0 to 122.9 ± 19.8 kU·L\(^{-1}\); \( p = 0.037^* \)) and PON1 concentrations increased 11.3% (from 109.5 ± 24.3 to 121.9 ± 19.8 ug·mL\(^{-1}\); \( p = 0.015^* \)) with the combination of exercise and niacin. The combination of
acute exercise and niacin therapy in men with MetS includes a modest increase in PON1 activity and PON1 concentration without observed changes in oLDL, MPO, or clinically-recognized measures of lipoprotein characteristics.
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CHAPTER I.
INTRODUCTION

Metabolic Syndrome

The metabolic syndrome (MetS) is characterized by a cluster of cardiovascular disease (CVD) risk factors that include abdominal obesity, dyslipidemia, hypertension, insulin resistance, and prothrombotic and proinflammatory states [1]. According to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII), an individual may be classified with MetS if they have three of the following risk factors: waist circumference ≥ 102 cm for men and ≥ 88 cm for women; triglycerides (TG) ≥ 150 mg·dL⁻¹; high density lipoprotein concentration (HDLc) ≤ 40 mg·dL⁻¹ for men and ≤ 50 mg·dL⁻¹ for women; systolic blood pressure > 130 and/or diastolic blood pressure > 85 mmHg, and; fasting plasma glucose levels ≥ 100 mg·dL⁻¹ [1]. It has been estimated that 1 out of 3 U.S. adults over the age of 20 can be classified as having MetS [2, 3].

Lipoprotein Particles

The atherogenic lipid profile that commonly occurs in MetS, includes small, dense low- and high-density lipoproteins [1, 4]. Individuals with MetS have increased activity of two important proteins that are involved with reducing the size of LDL particles and increasing their density; cholesterol ester transfer protein (CETP) and hepatic lipase (HL) [1, 5, 6]. CETP removes cholesterol ester from LDL in exchange for triglyceride (TG), making the LDL particle smaller. Then, HL removes some TG from LDL which further reduces the size of the lipoprotein [7]. Additionally, the increased activity of CETP will enrich the HDL particle with TG [8]. These exchanges result in compositional
changes in the HDL particle from a large, less dense HDL particle to a small, dense HDL particle [9]. These small, dense lipoprotein subfractions may increase CVD risk by reducing endothelial integrity, exacerbating oxidative stress and arterial inflammation, and contributing to the development of atherosclerosis [1, 5, 6, 10].

**Inflammation and Oxidative Stress in Relation to Metabolic Syndrome**

Small, dense LDL particles have a decreased affinity for the normal LDL receptor which prolongs their presence in circulation and makes them more prone to oxidation [1, 5, 6, 11, 12]. Oxidized LDL stimulates the expression of endothelial adhesion molecules [13, 14]. The increased expression of adhesion molecules on the endothelium potentiates the entrance of circulating monocytes and small, dense LDL into the arterial wall [15]. The interactions between monocytes, which become macrophages, and LDL that have crossed the endothelial lining and are in the vascular wall contribute to the atherosclerotic plaque accumulation and inflammation. In addition, vascular inflammation and the accompanying oxidative stress dampen nitric oxide production, and induce endothelial dysfunction, and a prothrombotic state [16-18].

HDL particles protect LDL against oxidative stress in peripheral tissues [9]. However, this protective mechanism is diminished in dyslipidemia and chronic inflammation [19]. Small, dense HDL particles are more prone to oxidative modification [20, 21]. A key mechanism for the oxidative modification of HDL could be a result of increased myeloperoxidase (MPO), which is a powerful oxidant released from neutrophils and phagocytes during oxidative stress and chronic inflammation [20-22]. The modification of HDL results in a decreased ability to promote cholesterol efflux from peripheral tissues, which contributes to further inflammation and the development of atherosclerosis [23, 24].
Paraoxonase 1

Oxidation of LDL and HDL can be attenuated by an important enzyme, paraoxonase 1 (PON1) [25-29]. This enzyme is in the paraoxonase (PON) family, which consists of three enzymes PON1, PON2, and PON3. However, PON1 is the most abundant [30] of the PON family of enzymes that circulates exclusively with the HDL particle [31, 32]. PON1 was first recognized for the ability to detoxify organophosphate compounds and its name was derived from a commonly used substrate, paraoxon [33-35]. More recently, studies have focused primarily on PON1’s antioxidative properties, which are inversely related to CVD [36]. PON1 is a calcium-dependent enzyme inhibited by ethylenediaminetetraacetic acid (EDTA) [36-39]. PON1 is synthesized and secreted by the liver [39]. HDL particles transiently associate with the hepatic cell membrane via scavenger receptor B1 (SR-B1) and remove PON1 from the liver [39]. Apolipoprotein A1 (Apo A1) is not necessary for the binding of PON1 to HDL; however, it is necessary for the stability and activity of the enzyme [40]. HDL is necessary to stimulate hepatic secretion and maintain normal levels of PON1 in circulation [41].

Regulation of Paraoxonase 1

As with other proteins, regulation occurs at many levels. The PON1 concentration and activity in serum is primarily determined by the status of PON1 gene expression in the liver [42]. Draganov et al. [43] and Yeung et al. [44] demonstrated transcriptional regulation of PON1 by substituting C42 or C353 with alanine, which decreased the secretion and activity of PON1. Furthermore, Osaki et al. [45] showed that over-expression of specificity protein 1 (Sp1) enhances PON1 promoter activity, while over-expression of protein kinase C (PKC) diminishes the promoter activity of PON1. Camps et al. [46] found that peroxisome proliferator-activated receptor-γ (PPAR-γ), also a transcription factor, increases PON1 expression and decreases oxidative
stress. Thus, PON1 transcription appears to be regulated by transcription factor Sp1 [45, 47, 48], PKC [45], and PPAR-γ [46].

**Actions and Mechanisms of Action of Paraoxonase 1**

PON1 inhibits the oLDL-stimulated production endothelial binding proteins, which suggests an early involvement of PON1 in the prevention of atherosclerosis [49]. In contrast, inhibition of PON1 will reduce HDL’s ability to prevent LDL oxidation and to prevent monocyte migration into the peripheral tissues [36]. PON1 may help to prevent or slow the atherosclerotic process by reducing the formation of oLDL and decreasing the uptake of oLDL by macrophages [38, 50-52]. Thus, PON1 helps to prevent the inflammatory process and oxidative stress involved in atherosclerosis [38, 39].

PON1 inhibits macrophage cholesterol biosynthesis and enhances macrophage cholesterol efflux via the ATP binding cassette transporter A1 (ABCA1) [31]. ABCA1 is a transporter that mediates the transfer of cholesterol and phospholipids to lipid poor apolipoproteins in reverse cholesterol transport [53]. The reduced formation and increased removal of cholesterol improves the antioxidant potential of the HDL particle [31].

Aviram et al. [26] suggests that PON1 protects HDL from oxidation by hydrolyzing oxidized cholesterol esters and phospholipids, preserving the antiatherogenic properties of HDL. In support of this hypothesis, Rosenblat et al. [54] demonstrated that the HDL from mice injected with PON1 was less prone to oxidation than compared to mice that were not injected with PON1.

Biologically, PON1 is effective at protecting the outer membranes of lipoproteins composed of phospholipids from the damaging effects of lipid peroxidation [55]. PON1 has peroxidase-like activity capable of hydrolyzing hydrogen peroxide, which is a major reactive oxygen species produced in conditions of oxidative stress [26]. This may be a
means by which PON1 prevents monocyte chemotaxis and adhesion to endothelial cells [56]. PON1 can act as a hydrolase enzyme, which allows it to hydrolyze oxidized lipids in oxidized LDL as well as prevent the formation of oLDL. The hydrolase activity of PON1 protects macrophages against oxidative stress by hydrolyzing lipids, inhibiting macrophage mediated oxidation of LDL, and decreasing the uptake of oxidized LDL by macrophages [31]. Rosenblat et al. [57] demonstrated that PON1 was able to prevent monocyte to macrophage differentiation, reduce monocyte chemotaxis, and adhesion to endothelial cells all of which may attenuate the development of atherosclerosis.

**Lifestyle Factors that Influence Paraoxonase 1**

There are a number of modifiable lifestyle factors that influence the activity of PON1 and one or more of the mechanisms described previously may be involved. The mechanisms by which lifestyle factors influence PON1 are not clear and each factor may alter the concentration and activity of PON1 by different or similar means. Aging [58], smoking [59], and high fat diet [60] are lifestyle factors that have been shown to negatively influence PON1 activity. Adults who are obese [61] or have coronary heart disease [62] have lower concentrations and activities of serum PON1. An age-related decrease of PON1 may be attributed to oxidative stress conditions and increased progression and severity of atherosclerosis with age [58]. The decrease in PON1 caused by smoking appears to be a result of increased oxidative stress [63, 64]. Moderate alcohol consumption enhances PON1 activity, while chronic alcohol consumption may dampen PON1 activity [65, 66]. Alcohol may modulate PON1 activity by affecting PKC, which may phosphorylate and regulate the binding of Sp1 to the promoter region of PON1 [45].
**Paraoxonase 1, Metabolic Syndrome, and Cardiovascular Disease**

Oxidative stress is typically associated with chronic disease like MetS and CVD [67-69]. Senti et al. [70] and Blatter Garin et al. [4] demonstrated that PON1 activity was lower in subjects with MetS. More recently, Martinelli et al. [71] found that subjects with MetS have lower PON1 activity as well as lower PON1 concentration than without MetS. PON1 activity is also lower in individuals with CVD. McElveen et al. [72] demonstrated that PON1 activity was lower in individuals with myocardial infarction (MI) than in controls. In addition, Navab and coworkers [73] concluded that individuals with high HDL, but low PON1 activity were more likely to present with CVD as compared to those with low HDL and high PON1 activity. Indeed, lower PON1 activity and concentration were observed at the time of a MI [74]. In contrast, higher PON1 activity was associated with a significantly lower incidence of cardiovascular events [75].

**Exercise**

The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recognizes the importance of therapeutic lifestyle changes to improve dyslipidemia and reduce the risk for cardiovascular disease [76]. It is accepted that regular physical exercise improves overall health and lipid profiles, thus, decreasing cardiovascular disease risk [77]. There appears to be a dose response relationship between exercise volumes and HDLc [78, 79]. Individuals who expend 700 to 3000 kcals/week may increase HDLc between 3.5 to 6 mg·dL⁻¹ as compared to sedentary individuals [78-80]. Acute bouts of exercise rather than exercise training can transiently improve lipid profiles of sedentary individuals. Sedentary individuals participating in acute aerobic exercise at an intensity of 70 to 80% maximal oxygen uptake (\( \dot{V}O_2 \text{ max} \)) and a caloric expenditure between 350 and 500 kcals have improved lipid profiles by increasing plasma HDLc and decreasing triglyceride concentrations [81-83].
Elevated oxygen consumption during exercise can produce excess free radicals and heightens the oxidative stress observed after strenuous exercise [84, 85]. More recently, research has focused on exercise-induced reactive oxygen species (ROS) production in the mitochondria contributing to localized oxidative stress. This exercise-induced ROS leads to oxidative damage through modification of lipids, proteins, and DNA [86-88]. Buettner et al. [89] suggest a hierarchy for free radical production during exercise leading to downstream lipid peroxidation. LDL particles become more susceptible to oxidation following intense aerobic exercise and the susceptibility persists up to four days following the exercise [90, 91].

On the other hand, PON1 activity is higher in active adolescents as compared to inactive adolescent [92, 93], and acute bouts of exercise transiently increase PON1 activity [94, 95]. However, these later two studies only included apparently fit adults and did not include individuals with MetS. Moreover, the volume for the aerobic exercise was not described in these two studies. As such, there is limited information on the effects of exercise and PON1.

**Niacin**

NCEP has identified three primary targets for reducing CVD risk: 1. reduce LDL cholesterol; 2. reduce non-HDLc, and; 3. increase HDL cholesterol [1]. Treating these abnormal lipids with lipid altering medications can reduce cardiovascular events by 30% [96]. Niacin appears to improve dyslipidemias by, reducing the number of small dense LDL particles [97], decreasing triglyceride (TG) concentrations [98], and increasing HDLc [99-102]. Niacin inhibits adipocyte lipolysis and contributes to a decrease in plasma levels of free fatty acids (FFA) [103, 104]. The lower levels of FFA results in a decreased production of TG and VLDL by the liver [105], which contributes to a
decreased breakdown of HDL and decreased accumulation of cholesterol ester in LDL particles [106].

Niacin increases the LDL particle size, which reduces the potential of LDL to infiltrate the vascular endothelium and decreases the risk of CVD [107, 108]. Pan et al. [97] demonstrated that extended-release niacin (500 mg to 4000 mg·day⁻¹) titrated over a period of one to four months, reduced small dense LDL concentrations by 43%, increased LDL particle size by 0.9 nm, and increased HDLc by 36% in dyslipidemic patients. Goldberg et al. [109] demonstrated that multiple doses of extended-release niacin (500mg to 3000 mg·day⁻¹) over 25 weeks was effective at increasing HDLc by 10% to 30% in a dose dependent fashion up to 2500 to 3000 mg·day⁻¹. The favorable increase in HDLc by extended-release niacin appears to be a result of its ability to increase HDL₂ subfraction [110]. The HDL₂ subfraction has antioxidant properties, inhibits thrombotic factors, and is considered to be more cardioprotective of the HDL subfractions [111, 112]. The increase in HDL₂ appears to be a sign of improved reverse cholesterol transport [97, 109]. However, the mechanism is not fully understood [113]. Sorrentino et al. [114] performed a randomized controlled study to assess extended released niacin therapy over 3-months and placebo on endothelial effects of HDL in type II diabetics. Niacin contributed to a marked improvement in the endothelial protective functions of HDL. Niacin was associated with increased endothelial nitric oxide and reduced superoxide production. Sorrentino et al. [114] also demonstrated that extended-release niacin increased the plasma HDLc by approximately 6 mg·dL⁻¹. Furthermore, extended-release niacin reduced oxidative stress by decreasing MPO activity as compared to placebo [114].
Combined Effects of Exercise and Niacin

Exercise and niacin therapy are prescribed to address health and reduce CVD risk in MetS [115]. Acute exercise and niacin therapy independently increase HDLc by different mechanisms. HDL is required for PON1 to be released from the liver and the increased HDL in circulation from the combined effects of acute exercise and niacin therapy could promote or induce a greater increase in PON1 over and above the independent effects. However, it is not known if these combined therapies favorably alter antioxidant functions of PON1. Our purpose was to characterize the independent and combined effects of acute exercise and extended release niacin on PON1 in men with MetS.

Research Hypothesis and Rationale

Question 1:

Does one session of exercise, at an intensity of 60 to 70% \( \dot{V}O_{2\text{max}} \) and resulting in a caloric expenditure of 500 kcals, influence the activity and concentration of PON1?

Hypothesis 1a:

It is hypothesized that an acute bout of exercise would increase the concentration and activity of PON1.

Rationale

Acute exercise increases PON1 activity immediately post-exercise and PON1 activity returns to pre-exercise levels within 2 hours in apparently fit individuals [94, 95]. The caloric expenditure of this exercise session was not reported and it remains to be determined if the volume of exercise may influence PON1. An acute bout of exercise expending between 350 and 500 kcals at an intensity of 70% to 80% \( \dot{V}O_{2\text{max}} \) increases plasma HDLc and decreases TG concentrations up to 48 hours following the exercise.
session [81, 82]. Since PON1 is an HDL-associated enzyme, the increases in plasma HDLc following an acute exercise session expending approximately 500 kcals may reflect changes in HDL characteristics that enhance the concentration and activity of PON1 in men with MetS 24 hours post exercise in spite of increased oxidative stress observed after exercise.

Question 2:
Does six weeks of niacin therapy, titrated from 500 to 1500 mg·day\(^{-1}\), influence the activity and concentration of PON1?

Hypothesis 2a:
It is hypothesized that six weeks of niacin therapy alone would increase the concentration and activity of PON1.

Rationale
Niacin action occurs at the liver causing a decreased assembly and secretion of VLDL [116]. Niacin also acts on the adipocyte where it decreases the release of free fatty acids [117]. Niacin has many anti-inflammatory effects. For instance, niacin reduces endothelial dysfunction by decreasing reactive oxygen species as well as increasing the removal of excess cholesterol via the ABCA1 receptor on the macrophage [103]. Furthermore, niacin decreases the number of small dense LDL particles [97]. Recent studies indicate niacin increases the mean particle size of LDL therefore reducing the potential for oxidative stress [107, 108]. Niacin reduces the enzymes that are responsible for the oxidation of LDL as well as inhibit the cytokines (e.g., c-reactive protein) and adhesion molecules (e.g., vascular cell adhesion molecule-1 and MCP-1) that contribute to development of an atherosclerotic lesion [103]. Sorrentino et al. [114] demonstrated that extended release niacin decreases markers of lipid peroxidation (e.g., MPO and malondialdehyde) as compared to placebo. This group
concluded the decrease in MPO appears to prevent the oxidation of HDL, but the mechanism by which niacin exerts these effects is unclear [114].

Statins increase PON1 activity by decreasing oxidative stress or by up-regulating liver PON1 expression [118]. If niacin has similar effects at reducing oxidative stress in the vasculature and liver, then it is possible to have similar effects of increasing PON1. Additionally, statins activate PPAR-γ in monocytes [119] and heart [120], which can increase expression of PON1 [120, 121]. Since niacin increases PPAR-γ in macrophages [122], then it is possible to have similar increases in PON1 as seen with statins. Furthermore, the actions of niacin occur at the liver where synthesis and secretion of PON1 occur, it may be possible by some unknown mechanism to influence changes in PON1 concentration and activity.

*Question 3:*
Does one session of exercise after six weeks of niacin therapy influence the concentration and activity of PON1 more so than exercise or niacin alone?

*Hypothesis 3a:*

It is hypothesized that a single exercise session after six weeks of niacin would increase the concentration and activity of PON1 to a greater extent than exercise or niacin alone.

*Rationale*

Aerobic exercise [81, 82, 123] and extended-release niacin [97, 109, 114] appear to independently ameliorate dyslipidemia. Acute exercise increases PON1 activity immediately following the exercise but returns to pre exercise levels within two hours [94]. However, it is not clear how niacin therapy would influence PON1. Niacin decreases the production of TG and VLDL by the liver, which result in a decreased clearance of HDLc. The decreased clearance of HDLc may provide an avenue to where additional PON1 can be removed from the liver. Niacin therapy also decreases markers
of oxidative stress in the endothelium [114]. This may provide a potential mechanism to influence a change in PON1. If exercise and extended release niacin work by different mechanisms, then it is possible to see a more pronounced increase in PON1 following the combination of exercise and niacin. Niacin could potentially modulate exercise-induced oxidative stress and increase PON1 activity combining the effects of exercise and niacin. Our understanding of PON1 regulation by niacin or by exercise is limited.

Limitations

- Physical activity was self-reported throughout the experimental period
- Only two sampling points were obtained with exercise. Baseline (immediately pre-exercise) and 24 hours post-exercise. Exercise samples were obtained prior to and after initiating 6 weeks of niacin therapy.

Delimitations

- Only physically-inactive, non-smoking, male participants with metabolic syndrome, -[1]-, and no contraindications to niacin therapy were recruited from the Auburn area
- Male participants with liver disease, on lipid lowering and/or glucose altering medications were excluded
- Only male participants between the ages of 35 and 65 were recruited
- Participants performed a single session of exercise at 60 to 70% \( \dot{V}O_{2\text{max}} \)
- Participants were prescribed extended release niacin that was titrated over a 3-week period from 500 mg·day\(^{-1}\) (week 1) to 1500 mg·day\(^{-1}\) (week 3 through 6).
**Significance of the Study**

PON1 is an important HDL-associated enzyme that has antioxidant properties. PON1 has the capacity to attenuate atherosclerosis and cardiovascular disease. Exercise appears to increase PON1 activity in apparently-healthy individuals; however, little data exists in individuals with MetS. This is the first study to investigate the relationship between PON1 and extended-release niacin. Statins have been found to increase PON1 through the activation of PPAR-γ. This is important because niacin can activate PPAR-γ, so it may be a potential mechanism to increase PON1. This study has been designed to examine the independent and combined influences of exercise and niacin therapy on PON1. Exercise and niacin may independently improve lipid profiles in individuals with dyslipidemia and their combined influence may impart additional health benefits related to lipid transport and oxidative stress. We anticipate that the results of this study will help us understand the clinical significance of exercise and niacin therapy for primary treatment of dyslipidemia and attenuating the development of atherosclerosis in those at higher risk for the development of CVD.
CHAPTER II.
REVIEW OF THE LITERATURE

Metabolic Syndrome

There are a number of organizations that have established criteria to identify those with MetS [1, 124, 125]; however, the NCEP ATPIII is the most widely used criteria in the United States. MetS is generally described as clustering of risk factors that include obesity, hypertension, insulin resistance (IR), and dyslipidemia [126-128]. MetS can lead to overt type II diabetes mellitus (T2DM) and CVD [126-128].

In 2002, the age-adjusted prevalence for both men and women was estimated to be 23 and 24% [129, 130]. More recently, Ervin et al. [2] and Ford et al. [3] estimated that approximately 34% of individuals over the age of 20 meet the criteria for MetS. Together these surveys suggest an increasing trend in MetS prevalence over the last decade.

Obesity is a major determinant of IR and MetS [131-133]. According to NHANES from 2009 to 2010, 33% of adult Americans were overweight [body mass index (BMI) of 25.0–29.9 kg/m²] and an even larger proportion (36%) were obese (BMI ≥ 30.0 kg/m²) [134]. The prevalence of hypertension, IR, diabetes, dyslipidemia, and CVD are higher in obese than in non-obese individuals [135-139]. However, body fat distribution, rather than obesity, appears to be a greater determinant of these risk factors. Specifically, it is the accumulation of fat in the abdominal region and around internal organs -clinically known as central fat distribution- that is implicated in cardiovascular and metabolic dysfunction [140, 141].
Moreover, all adipose tissue is not the same. Differentiation of cells within the adipose tissues leads to a change in the function of adipose tissue in response to hormones and growth signals [142-145]. It is this differentiation within accumulating central fat that is thought to lead to chronic low-grade inflammation, abnormal adipokine secretion, and disturbances in fibrinolysis, which can result in oxidative stress, IR, endothelial dysfunction and atherosclerosis [6, 142-145].

Approximately 36% of individuals that are obese have IR and nearly 50% of individuals diagnosed with MetS have IR [146-148]. IR also exists in the general population in the absence of obesity [149]. Other risk factors, such as hypertension, diabetes, stroke, and abnormal lipid metabolism, cluster with IR [150]. This contributes to the reason the European Group for the Study of Insulin Resistance support IR as the primary contributor to MetS rather than central obesity [151].

Lifestyle factors such as physical inactivity and poor diet contribute to the development of central obesity and IR. The debate regarding central obesity or IR holding primary importance in the development of MetS rages on. However, central obesity and IR largely result from a sedentary lifestyle and poor diet and both contribute to MetS and the atherogenic dyslipidemia [139].

**Dyslipidemia**

Dyslipidemia is a term used to describe any number of combinations of lipid abnormalities. The dyslipidemia associated with MetS is noted by an elevation in plasma TG $\geq 150$ mg·dL$^{-1}$ and cholesterol $\geq 200$ mg·dL$^{-1}$ along with low levels of plasma HDLc $\leq 40$ mg·dL$^{-1}$ for men and $\leq 50$ mg·dL$^{-1}$ for women [1]. Dyslipidemia has been identified as a major risk factor for development of coronary heart disease and is a leading cause of death among men and women in the United States [152-154].
In the MetS, there is predominance of small LDL particles that result directly from the overproduction of TG. The increased TG concentrations seen in individuals with IR and hyperinsulinemia can be largely attributed to increased circulating levels of free fatty acids [155]. The increased free fatty acids are taken up by the liver, and contribute to increased hepatic TG synthesis and production of very-low-density lipoprotein (VLDL) [156-158]. The overproduction of VLDL resulting from IR can lead to proatherogenic alterations in HDL and LDL particles [159, 160]. The common traits of this dyslipidemia are increased TG, decreased HDL, and increased numbers of small, dense LDL particles [1].

The small, dense LDL particles have a decreased affinity for the LDL receptor, which prolongs their presence in plasma [161, 162] and increase their ability to penetrate the vascular endothelium [163]. Once in the vascular endothelium, small, dense LDL is modified to form oLDL [163]. The oLDL is taken up by activated macrophages and is thought to lead to the formation of fatty streaks and atherosclerotic lesions in the arterial vasculature [163].

In addition, cholesterol ester transfer protein (CETP) activity is increased as TG levels continue to rise. This increased activity results in the exchange of cholesterol esters and TG among lipoprotein fractions [164]. Increased CETP contributes to the development of small, less dense HDL, which reduces the ability of HDL to promote reverse cholesterol transport [165].

**HDL Oxidation**

HDL is involved in preventing atherosclerosis by removing cholesterol from peripheral tissues and macrophage foam cells in the arterial wall [166, 167]. The antioxidant properties of HDL may become impaired during atherosclerosis in the presence of myeloperoxidase (MPO). MPO appears to be one mechanism by which
HDL becomes oxidized and loses its antiatherogenic properties [20, 21]. MPO is released from phagocytes that respond to areas that have chronic inflammation [22] and MPO uses hydrogen peroxide (H$_2$O$_2$) and chloride (Cl$^-$) to form hypochlorous acid (HOCl), a powerful oxidant [20]. Bergt and colleagues [20] exposed HDL and lipid-free Apo A1 to HOCl, which caused both HDL and Apo A1 to become oxidized. The oxidation of HDL and Apo A1 by HOCl decreases its ability to promote cholesterol efflux by means of the ABCA1 pathway and inhibits the function of HDL during atherosclerosis [20].

Paraoxonase 1

Mazur et al. [33], in 1946, was the first to demonstrate the presence of an enzyme in the plasma and tissue of rabbits and humans which was capable of hydrolyzing organophosphate (OP) compounds. In the early 1950’s, Aldridge et al. [34, 35] investigated another OP that led to the initial identification of PON1 as “A-esterase” for its ability to hydrolyze phenylacetate. PON1 was originally investigated for its ability to hydrolyze OP insecticides such as parathion which is metabolized to paraoxon [33-35]. The name paraoxonase is derived from paraoxon, the first OP substrate to be hydrolyzed by this enzyme [35]. In the early 1990’s, Mackness et al. [52] were the first to link PON1 to CVD. Mackness provided this initial link with CVD by demonstrating that PON1 could prevent the accumulation of lipoperoxides in LDL [52].

PON family consists of three enzymes: PON1, PON2, and PON3. [33-35]. PON1 is the most abundant [30] of the PON family enzymes associated with HDL [31] and contributes to the antioxidant potential. PON1 is located on chromosome 7 between q21.3 and q22.1 in humans, and has a weight of 43 kDa [30, 168], and the structure is a six-bladed b propeller [169]. PON1 is a calcium-dependent enzyme which is synthesized and secreted by the liver and is associated with the HDL particle [37-39, 170]. HDL is believed to transiently bind to the hepatocyte via the scavenger receptor
B1 (SR-B1), where PON1 is transferred to HDL [39, 171]. The hydrophobic N-terminal leader sequence of PON1 anchors PON1 to the HDL particle [28]. Even though, Apo A1 is not necessary for the association of PON1 with HDL or phospholipids [28]. However, the stability and activity of PON1 is enhanced in the presence of Apo A1 as compared to without Apo A1 [28]. Gaidukov et al. [172, 173] examined the ability of Apo A1, Apo AII, Apo AIV, Apo E3, and Apo E4 and their ability to stabilize and bind PON1 to HDL. These investigators [172, 173] found that Apo A1 were more effective at binding and stabilizing PON1 to the HDL particle. Furthermore, they noted that the presence of Apo A1 enhanced the antioxidant activity of PON1 to prevent oxidation of LDL [172, 173].

PON1 is effective at protecting the outer membranes of cells from being damage by the products of lipid peroxidation and plays a central role in the inhibitory effect of HDL on lipid peroxidation [25, 55]. The action of PON1 assists HDL in protecting against atherosclerosis by preventing oxidation of LDL [50]. PON1 attenuates oxidation of lipoproteins as well as formation of foam cells, assists in removal of lipid peroxides, and prevent atherosclerosis [31, 51].

PON1 reduces other atherosclerotic processes such as oxidative stress and adhesion molecule expression both of which can be induced by macrophages. PON1 helps maintain the vascular integrity and decreases CVD risk, thereby protecting the vasculature from the development of an atherosclerotic lesion. Shih et al. [174] observed a link between PON1 and heart disease in mice, when fed a high-fat, high cholesterol diet. They found HDL from PON1 knockout (KO) mice couldn’t prevent LDL oxidation in a model simulating the wall of an artery. Furthermore, the macrophages of these PON1 KO mice contained more oxidized lipids, which are more prone to oxidation of LDL. Shih and colleagues [174] concluded that the PON1 KO mice were more likely to develop an atherosclerotic lesion as compared to their wild type counterparts as well as increased oxidative stress [174]. Similarly, Ng et al. [175] observed that PON1 KO
mice showed a significant increase in leukocyte adhesion and aortic adhesion molecules P selectin and ICAM1 mRNA as compared to the wild type mice. In addition, Rozenberg et al. [176] observed that PON1 KO mice significantly increased lipid peroxides, increased oLDL, and increased peroxide content on the macrophage as compared to the control mice. Rozenberg and colleagues [176] observed a significant increase in the production of superoxide anion from the macrophages in the PON1 KO mice. The release of superoxide anion increases the oxidation of LDL by macrophages [177]. Ng et al. [175] and Rozenberg et al. [176] both found PON1 deficiency would result in arterial and macrophage oxidative stress and increased endothelial adhesion molecules, which are processes that can lead to arterial plaque formation.

Our knowledge of PON1 has largely resulted from cell culture and animal research. However, it is possible and highly likely that the decreased PON1 activity in individuals with MetS and CVD may result from the formation of oLDL due to the interaction between oxidized lipids (i.e., oxidized phospholipids, oxidized cholesterol esters, and lysophosphatidylcholine) and free sulfhydryl groups of PON1 [178].

As with under-expression, over-expression of PON1 may also lead to less development of atherosclerotic lesions. PON1 has been shown to reduce the formation of oxidized LDL as well as decreasing the uptake of oxidized LDL by macrophages [38, 50-52]. Furthermore, Tward et al. [179] demonstrated that PON1 Transgenic (Tg) mice that were fed a high fat diet, showed a significant decrease atherosclerotic lesion size as well as a reduction in the monocyte chemotactic protein 1 (MCP-1) as compared to PON1 KO mice. The HDL that was isolated from the PON1 Tg mice had a significantly higher PON1 activity as compared to the PON1 KO mice, which suggest PON1 Tg mice were more effective at preventing oxidation of LDL. They found that a moderate increase in PON1 concentration protects against early and late stage lesion formation in PON1 Tg mice as compared to PON1 KO mice.
PON1, MetS, and CVD

A recent meta-analysis conducted by Zhao et al. [180] determined an association between PON1 activity and CHD. The meta-analysis involved 43 studies and included 9481 CHD subjects and 11,148 control subjects [180]. The researchers determined a standard mean difference of PON1 activity and CHD to be -0.78 [95% Confidence Interval (CI); -0.98 to -0.57; \( p < 0.001 \)]. According to Cohen’s definition [181], this finding represents a medium effect size and associates a decreasing PON1 activity as a risk factor for developing CHD [180]. Wang et al. [182] conducted a meta-analysis of 47 studies with 9853 CHD subjects and 11,408 control subjects. The researchers expressed the difference in mean PON1 activity and control as a ratio of the mean (RoM). The RoM was calculated by dividing mean activity of the case by the mean activity of the control, which allows for pooling of data from different studies as a percentage. The RoM for the overall meta-analysis was 0.81 [95% CI; 0.74-0.89; \( p < 0.00001 \)], and may be interpreted as an association of low PON1 activity with CHD. The pooled analysis indicated that patients with CHD had a 19\% lower PON1 activity than the controls. Both of these meta-analyses observed an increased risk of CHD in subjects with low PON1 activity regardless of age [180, 182].

MetS is closely associated with chronic inflammation and oxidative stress, which is characterized by increased production of cytokines and acute phase reactants [67-69]. Senti et al. [70] recruited 1364 men and women with and without MetS to investigate PON1 activity and lipid peroxidation. Senti and colleagues [70] established that 285 (153 men, 132 women) of the 1364 subjects met 3 of the 5 NCEP criteria for MetS. Participants in this investigation were stratified into groups based on the number of metabolic abnormalities (from 0 to 5), and PON1 activity and lipid peroxidation were compared between groups in a general linear model. They found that individuals with MetS had a significantly lower PON1 activity and higher lipid peroxidation than subjects
without MetS. Senti and colleagues [70] determined the low PON1 along with high lipid peroxidation could reflect an increased oxidative stress in individuals with MetS. This increased oxidative stress may compromise the protective functions of PON1.

Blatter-Garin et al. [4] recruited 773 men and women to investigate PON1 activity, PON1 concentration, and small, dense lipoprotein particles in individuals with and without MetS. The modified World Health Organization (WHO) criteria were used to classify 139 of the 773 individuals with MetS. Glucose intolerance was taken as fasting glucose ≥ 110 mg/dL or presence of type 2 diabetes. Ratios of LDL:ApoB and HDL:Apo A1 served as surrogates to LDL and HDL particle size. They found that individuals with MetS had a significantly smaller LDL and HDL particles than those without MetS. Individuals with MetS had significantly lower PON1 concentration and activity as compared to those without MetS. Overall interpretation of the findings were that those with lower PON1 activity and/or PON1 concentration are more susceptible to LDL and HDL oxidation [4].

More recently, Martinelli et al. [71] recruited 293 subjects, with and without MetS, to investigate PON1 activities in those with or without angiographically confirmed CAD. There were 195 of the 293 subjects with diagnosed CAD. Eighty-eight of the 293 subjects met the NCEP criteria for having MetS. As with previously discussed investigations, individuals with MetS had a significantly lower PON1 activity as compared to without MetS. Relative Odds Ratio (OR) for CAD was calculated in subjects with and without MetS. The authors concluded individuals with MetS and low PON1 activity had the highest risk for CVD (OR 4.34; 95% CI 1.44 – 13.10; p < 0.01).

McElveen et al. [72] recruited 383 subjects to investigate PON1 activity in individuals with and without previously diagnosed myocardial infarction (MI). McElveen and colleagues [72] found that PON1 activity was significantly lower in individuals who had an MI as compared to those who had not. They found the low PON1 activity was
associated with an MI, but could not determine if the low PON1 occurred before or after the MI [72].

Navab et al. [73] found that individuals with normal HDLc, but low PON1 activity, were more susceptible to coronary heart disease (CHD) than individuals with normal HDL and a significantly higher PON1 activity. They found that PON1 activity was lower in individuals with CHD as compared to those without.

Ayub et al. [74] recruited 50 subjects with diagnosed MI by electrocardiogram (ECG) and chest pain to investigate PON1 activity and PON1 concentration. They found PON1 activity and concentration were significantly lower in individuals with MI as compared to controls and the decrease in PON1 activity was attributed to a decrease in PON1 concentration. It could not be determined if the decrease in PON1 activity and concentration occurred prior to or after the MI.

In the Caerphilly Prospective Study [183], 1353 men aged 49 to 65 years were monitored for coronary heart disease (CHD) events for a mean period of 15 years. The PON1 activity of the 163 participants who experienced a coronary event was 20% lower than the men who did not. Quintiles comparing PON1 activity to CHD risk showed an inverse, graded relationship with the median change in the odds ratio across each quintile. Men in the highest PON1 quintile (highest PON1 activity) had decreased chance of having a coronary event (OR = 0.57, 95% CI .33 – .96) as compared to the lowest PON1 quintile (OR = 0.87; 95% CI .77 – .98) ($p = 0.039$). The results indicated that PON1 activity was an independent predictor of a new coronary event in men with preexisting CHD or at high risk for CHD [183].

More recently, Bhattacharyya et al. [75] recruited 1399 subjects to investigate the relationship between PON1 activity and systemic measures of oxidative stress and CVD risk. They found a significant association between low PON1 activity and increased markers of oxidative stress. The incidence of a cardiac event was significantly lower in
the highest quartile for PON1 activity (7.3%) as compared to the lowest quartile for PON1 activity (7.7%). Furthermore, adjusted hazard ratios between these two quartiles was 3.4 (95% CI, 2.1-5.5; \( p < .001 \)), which indicates that individuals with low PON1 activity are at increased CVD risk [75]. Taken together, the results herein demonstrate consistent evidence that low PON1 concentrations and decreased PON1 activity are independent predictors of CVD, even after adjusting for pre-existing CVD, age, and gender [184].

**Regulation of PON1**

PON1 expression is regulated by specificity protein-1 (Sp-1) through an interaction with protein kinase C (PKC), peroxisome proliferator activated receptor-gamma (PPAR-\( \gamma \)), and sterol-responsive element binding protein-2 (SREBP-2) (Figure 1) [45-47].

The PON1 gene is regulated by Sp1. Sp1 acts through the promoter GC boxes to up- or downregulate genes [185]. Sp1 activities are increased during maturation of monocytes into macrophages and during adipocytes differentiation [186]. Chronically high glucose concentrations are a key factor that activates Sp1 through an interaction with protein kinase C (PKC) in liver cells [187].

PPAR-\( \gamma \) is expressed highest in adipose tissues (white and brown adipose tissue) and activated macrophages, including the foam cells of atherosclerotic lesions [188-191]. PPAR-\( \gamma \) regulates the proliferation and differentiation of adipose cells [192], production of monocyte cytokines [193], and glucose homeostasis [194]. The presence of macrophage-colony stimulating factor (M-CSF) and oLDL can activate the expression of PPAR-\( \gamma \) in macrophages and monocytes [195]. Activation of PPAR-\( \gamma \) results in improved insulin sensitivity and enhanced glucose disposal in adipose tissue and skeletal muscle [196]. The mechanism in which activation of PPAR-\( \gamma \) improves insulin
sensitivity in adipose tissue may be by altering the production of cytokines (e.g., increase adiponectin, decrease tumor necrosis factor alpha, decrease resistin, and decrease MCP-1) [194, 197].

SREBP-2 modulates the transcription factors of several genes involved in cholesterol synthesis and receptor mediate uptake of cholesterol [198, 199]. SREBP-2 processing is mainly controlled by the cellular content of sterol [200, 201]. In the presence of high cholesterol, SREBP-2 exists in the endoplasmic reticulum as an inactive precursor. Low cholesterol levels will cause SREBP-2 to move from the endoplasmic reticulum (ER) to the Golgi apparatus, where SREBP-2 becomes biologically active and initiates cholesterol synthesis [200].

Figure 1 – Regulation of PON1.

PON1, Paraoxonase 1; Sp1, Specificity protein 1; PKC, Protein Kinase C; PPAR-γ, peroxisome proliferator activated receptor gamma; SREBP-2, Sterol-responsive element binding protein 2; AhR, aryl hydrocarbon receptor; MAPK, mitogen-activated protein kinase; dashed lines, indicate interactions; solid lines, direct activation.
**PON1 Activity and Concentration**

In general, PON1 activity is directly related to PON1 concentration [202]. Seres and colleagues [58] recruited 129 apparently healthy adults between the ages of 22 and 89 to examine the effects of aging on PON1. However, factors associated with increased oxidative stress can uncouple the relationship between PON1 activity and PON1 concentration [58].

**Mechanisms for Prevention of Oxidation by PON1**

Normal activity levels of PON1 have unique features to prevent the formation of an atherosclerotic plaque by inhibiting expression of MCP-1 [179, 203], inhibiting superoxide anion release [176], reducing macrophage cholesterol biosynthesis (MCB) [203-205], and promoting cholesterol efflux (Figure 2) [206, 207].

**Figure 2 – PON1 inhibits foam cell formation.**

Paraoxonase inhibits macrophage foam cell formation and attenuates atherosclerosis. Macrophage cholesterol accumulation can result from increased uptake of oxidized LDL, increased cholesterol biosynthesis, and reduced HDL-mediated macrophage cholesterol efflux. HDL-associated PON1 can inhibit the influx of cholesterol by oLDL into macrophages by different mechanisms: (1) hydrolysis of macrophage oxidized lipids, (2)
reducing macrophage-mediated oxidation of LDL, (3) reducing oLDL levels by hydrolysis of oxidized lipids in oxidized LDL, and (4) reducing oLDL uptake via the macrophage CD-36 scavenger receptor, secondary to cellular oxidized lipid (in the receptor area) hydrolysis. HDL-associated PON1 also inhibits cholesterol biosynthesis and enhances HDL-mediated cholesterol efflux via the ATP binding cassette transporter A1 (ABCA1). CE, cholesteryl ester; UC, unesterified cholesterol; PL, phospholipids; oLDL, oxidized LDL; CL-OOH, cholesteryl linoleate hydroperoxides; PL-OOH, phospholipid hydroperoxides. Permission to re-use. [31]

Endothelial MCP-1 expression is regulated by the formation of oLDL, which is a key component of the monocyte recruitment and initial stage of atherosclerosis [18]. LDL must be in close proximity to the arterial wall in order for LDL oxidation to occur [208, 209]. Once the phospholipids of LDL have been oxidized, macrophages can take up LDL at the scavenger receptors CD36 and SR-A, which can lead to development of foam cells in the arterial wall [208, 209].

PON1 is capable of preventing the formation of oLDL and the recruitment of monocytes by inhibiting MCP-1 expression [49, 179, 203]. Mackness et al. [49] used cultured human endothelial cells to investigate whether PON1 could suppress oLDL induced expression of MCP-1. The researchers found that adding oLDL to the endothelial cells significantly increased MCP-1 expression as compared to control. In contrast, the investigators added purified PON1 along with oLDL to the endothelial cells, which completely attenuated the secretion of MCP-1 as compared to the control. The ability of PON1 to inhibit MCP-1 secretion could be a result of decreased formation of oLDL caused by PON1. This could be a potential mechanism by which PON1 is antiatherogenic.

PON1 inhibits the macrophage from releasing superoxide anions, which results in a decreased oxidation of LDL [176]. Mouse peritoneal macrophages (MPM), used from the PON1<sup>0</sup> mice, were shown to readily activate NADPH oxidase to produce superoxide anions in the arterial wall as well as in macrophages [210]. The activation of
NADPH oxidase will increase the amounts of superoxide anions, which leads to an increased cell-mediated oxidation of LDL [176]. The PON1⁰ mice also exhibited increased production of lipid peroxides by 84% as compared to control [176]. Furthermore, the PON1⁰ mice also exhibited a 50% increased superoxide anion release as compared to the control. In contrast, the mouse peritoneal macrophages of the PON1Tg mice demonstrated lower oxidative stress and lower total lipid peroxides [176]. The macrophages from PON1Tg mice were less able to release superoxide anion and oLDL as compared to control [176]. They demonstrated that HDL-associated PON1 protected macrophages from oxidative stress as well as showing that apo-A1 improves PON1 protection against macrophage oxidation [176].

In the macrophage, PON1 prevents the formation of cholesterol. This results in a reduced amount of excess cholesterol that needs to be removed [203-205]. Macrophage cholesterol biosynthesis is stimulated by means of oxidative stress. The rate limiting enzyme of this pathway is HMG-CoA reductase, which converts HMG-CoA to mevalonate [204]. However, HMG-CoA reductase inhibitors were shown to inhibit cholesterol synthesis in the liver and macrophages [205]. PON1 has been shown to have a similar inhibitory effect on macrophage cholesterol biosynthesis [203]. Rozenburg et al. [203] demonstrated that macrophage cholesterol biosynthesis is inhibited by PON1 downstream of mevalonate by showing an accumulation of lanosterol. The researchers [203] used PON1⁰ MPM that showed a 50% increase in MCB rate. Similarly, they incubated PON1⁰ MPM with purified human PON1 (hPON1) which inhibited macrophage cholesterol biosynthesis up to 84% [203].

PON1 works synergistically with HDL to promote cholesterol efflux from the macrophage [206, 207]. Cholesterol efflux from macrophages results in the excess removal of cholesterol by HDL and returned to the liver for degradation. Of the three HDL subfractions, HDL₃ has the highest PON1 activity and HDL₃ is very important in
reverse cholesterol transport [211, 212]. Rosenblat et al. [206] investigated cholesterol efflux by isolating HDL from human PON1\(^0\) mice and human PON1\(^{Tg}\) mice and measured the activity of PON1. The activity of PON1 was 2.4\% higher in the HDL-PON1\(^{Tg}\) as compared to control and there was no PON1 activity for the HDL-PON1\(^0\) [206]. The human HDL without PON1 reduced macrophage cholesterol content by 21\%. However, human HDL along with PON1 results in a 46\% reduction in macrophage cholesterol content. The investigators observed that PON1 stimulation of HDL-mediated macrophage cholesterol efflux results in a significant decrease in the macrophage cholesterol content by increasing lysophosphatidylcholine (LPC). LPC promotes HDL binding to macrophage and increases cholesterol efflux [36]. In a subsequent study, Rosenblat and colleagues [212] compared PON1 in lipoprotein deficient serum (LPDS) with PON1 in HDL to induce macrophage cholesterol efflux. The researchers [212] suggest that PON1-association with HDL is important for the anti-atherogenic effects of PON1. The dissociation of PON1 from HDL to lipoprotein-deficient serum (LPDS) in diabetic subjects reduces the anti-atherogenic properties of PON1. They found that PON1-associated HDL reduced lipid peroxidation by 33\% as compared to PON1 with LPDS. These results indicate that HDL-associated PON1 protects against lipid peroxidation more so than lipoprotein-free PON1 in LPDS. Furthermore, HDL-associated PON1 is 3.5 times more effective at promoting macrophage cholesterol efflux than lipoprotein-free PON1 in LPDS [212]. Berrougui et al. [207] loaded cholesterol and purified PON1 at varying concentrations of 5, 10, and 20 U·mL\(^{-1}\), which increased HDL\(_3\) mediated cholesterol efflux from the macrophage-like cells by 20.8\%, 24\%, and 63\%, respectively. In addition, PON1 significantly increase cholesterol efflux in macrophages that were over expressed with ABCA-1 receptor as compared to control macrophages. The research team interpreted their results as demonstrating a significant synergistic
effect on cholesterol efflux when adding varying concentrations of PON1 (5, 10, and 20 U·mL⁻¹) and Apo A1 to macrophage-like cells as compared to only adding PON1 [207].

In summary, the research by Mackness [49], Rozenburg [203], Rosenblat [212], and Berrougui [207] may be interpreted as support for the anti-atherogenic properties of PON1. Macrophage oxidation of LDL and foam cell formation are important factors in the early developmental stages of atherosclerosis. The ability of PON1 to attenuate macrophage foam cell formation and atherosclerosis is related to PON1 effects on expression of MCP-1, oLDL uptake, cholesterol biosynthesis, and cholesterol efflux. PON1 plays a pivotal role in the protection against oxidative stress and CVD.

Physical Activity

Go and colleagues [213] reported that 32% of adults over the age of 18 do not participate in leisure time physical activity. Physical inactivity was higher among women than men, 33.2% vs. 29.9%, respectively [213]. American College of Sports Medicine (ACSM) and American Heart Association (AHA) guidelines for adults aged 18-65 should accumulate a minimum of 30 minutes of moderate-intensity aerobic exercise on 5 days per week. The guidelines are minimum requirements for preventing disease associated with physical inactivity. However, according to the ACSM and Centers for Disease Control and Prevention (CDC), nearly half of all adults do not meet the minimum requirements of physical activity for improving and maintaining health [214, 215].

Health benefits of regular exercise include decrease blood pressure (BP), improved insulin sensitivity, improved glucose regulation, decreased body weight, and improve dyslipidemias [82, 123, 216-218]. Aerobic exercise improves lipid profiles by increasing plasma HDLc [82, 123, 216-218] and lowering plasma TG levels [82, 219]. Exercise may also enhance lipoprotein particle sizes [220]. The risk for developing MetS and T2DM is inversely associated with regular exercise [221-227]. Furthermore,
individuals with T2DM that regularly exercise have a decreased risk for developing cardiovascular disease [77].

Lifestyle interventions that include weight loss and exercise are very important in preventing T2DM. For example, the Finnish Diabetes Prevention Study (FDPS) [228] included 522 overweight individuals. The goal of this study was to evaluate lifestyle modification in order to prevent or delay onset of T2DM. Subjects were randomly placed into intervention group or control group. The control group only received oral and written instruction regarding diet and exercise and no individualized plan. The intervention group received an exercise plan, dietary plan, as well as an individualized help for exercise training during their participation. They found that lifestyle modifications regardless of group can reduce the incidence of diabetes by almost 60% [228].

The Diabetes Prevention Program (DPP) [229] included 3234 men and women. The goal of the study was to have subjects lose 7% of their body weight and exercise 150 minutes a week. The lifestyle intervention group BMI (kg·m⁻²) for 22 to <30 and 30 to <35 was 63% and 53% lower than the metformin group, respectively. Lifestyle intervention group had a 39% lower incidence of T2DM as compared to metformin group. Fasting plasma glucose levels in the range of 95 – 109 mg·dL⁻¹ were 48% lower in the lifestyle intervention groups as compared to the metformin group. Similarly, the fasting plasma glucose levels in the range of 110 – 125 mg·dL⁻¹ were 30% lower in the lifestyle intervention groups as compared to the metformin group. The results indicate that lifestyle modification was more effective at reducing the incidence of T2DM than metformin [229].

The HERITAGE Family Study [230] included 621 sedentary African Americans and Caucasians. Thirty-two of the one hundred and five participants classified with MetS showed significant improvements in HDL (increased 16%), lower TG (down 43%), lower BP (decreased 43%), lower fasting plasma glucose (38%), and decreased waist
circumference (decrease 28%) following the 20 week aerobic exercise training program. These thirty-two subjects were no longer classified with MetS according to the NCEP ATPIII criteria [230]. Lifestyle modifications (physical activity and weight control) can play a critical role in the prevention and treatment of MetS.

**Exercise and PON1**

The literature on the relationship between physical activity and PON1 is limited [92-95, 231, 232]. The proposed mechanism by which acute exercise increases PON1 is through the production of oxidative stress, in particular oLDL. Furthermore, exercise-induced oxidative stress occurs as a result of increased production of reactive oxygen species [85]. The proposed mechanism for PON1 against oxidative stress appears to be an interaction between reactive oxygen species and the free sulfhydryl group at cysteine-284 [178, 233]. The antioxidant activities of PON1 are increased to counter the effects of the free radicals produced on lipoproteins [94, 95]. However, the exact mechanism is not fully understood.

**Cross-Sectional Studies**

Cross-sectional data shows higher PON1 activity in adolescent athletes as compared to their non-athletic counterparts (Table 1) [92, 93]. The exercise intensity for the cross-sectional studies was moderate- to high-intensity. The frequency and amount of the exercise varied from 3 to 6 times per week and 1.5 to 2 hours of exercise for each session. The investigators for each study utilized different forms aerobic exercises as well as endurance and strength training exercise. Following a thorough literature search, there were only two cross-sectional studies involving exercise and both studies involved adolescent individuals. These cross-sectional studies should be interpreted
with care because we do not know if some or all of the outside influences [e.g., body weight, diet, smoking habits, etc.] on PON1 were taken into consideration.

Cakmak et al. [92] investigated PON1 activity and oxidative stress in 64 adolescent athletes (57 boys and 7 girls) that played basketball and 32 adolescents in the control group (24 boys and 8 girls) that didn’t participate in sports. The physical characteristics for both groups were not reported. The food intake was similar in both groups. The basketball regimen consisted of 3 practice sessions totaling 6 hours of accumulated exercise a week. The control group did not participate in sports. In addition to PON1 activity, they tested each participant for total anti-oxidative capacity, total peroxide concentration, total oxidant status, oxidative stress index, and lipid profiles. All of the oxidative stress markers were significantly higher in the basketball group than the control group. PON1 activity was higher in adolescent boys that played basketball (177.3 U·L⁻¹) as compared to the control (98.1 U·L⁻¹). Increased PON1 activity was in response to physical activity and PON1 has a cardioprotective effect in adolescents who participate in basketball [92].

Hamurcu and colleagues [93] recruited 18 male adolescent boys that participated in wrestling and 18 sedentary males in the control group. The purpose of the study was to compare PON1 activity in adolescent male wrestlers with the sedentary control group. The wrestling group trained 6 days per week accumulating a total of 9 hours exercise per week. The control group was comprised of adolescents that were sedentary. The age and BMI of both groups were similar. Dietary information was not reported for either group. Hamurcu et al. [93] demonstrated that wrestlers had a higher PON1 activity (334.89 U·L⁻¹) than the control (274.74 U·L⁻¹). They found that using wrestling as a form of exercise can increase PON1 and this increase was associated with a reduction in oxidative stress.
In summary of cross-sectional studies, the data indicate that adolescent individuals that participate in physical activity on three or more days per week at a moderate to high-intensity exercise have a higher PON1 activity than those who do not participate in physical activity. These cross-sectional studies only included adolescents. Therefore, more investigations with adults are needed to validate the higher PON1 levels seen in adolescent that are physically active.

**Training Studies and PON1**

To date five training studies have been conducted to evaluate PON1 in both men and women (Table 2) [94, 231, 232, 234, 235]. These studies that examined PON1 activity in response to chronic exercise have varying results. These training studies
included subjects that led a sedentary lifestyle, known MetS, and known CAD. The interventions ranged from 12 to 28 weeks in duration. The exercise sessions were 3 to 5 days per week at an intensity between 70 and 85% heart rate (HR) maximum. Each exercise session lasted between 40 to 50 minutes. The contradictory results could be attributed to differences associated with exercise interventions and the subjects selected for participation.

Tomás et al. [94] followed 10 women and 7 men as they participated in a 16-week training program. During the first 8 weeks, the participants were asked to perform aerobic exercises (e.g., running, swimming, and aerobic games) on four days per week and for a duration of 30 minutes per session. The frequency and duration was increased to 5 days per week and 50 minutes per session for the remainder of the study. Training did not increase PON1 activity (253 U·L⁻¹) as compared to before (258 U·L⁻¹) the training session [94].

Richter et al. [232] investigated the effect of 12 weeks of exercise training with 32 adults (18 men and 14 women) that were at elevated risk or having CVD. The participants exercised 3 to 5 days per week for 40 minutes at 70 to 80% maximum HR by either walking or running. They demonstrated exercise treatment had no influence on PON1 activity. In contrast, Goldhammer et al. [231] had 37 participants which included both men and women. All participants were diagnosed with coronary artery disease (CAD). Each participant was asked to exercise 3 times per week at 70 to 85% of peak heart rate over a 12 week period. PON1 activity was increased 16.7% following the 12 weeks of training.

Casella-Filho et al. [234] investigated the effects of 12 weeks of exercise training on HDL and HDL functional characteristics in individuals with MetS. Participants exercised 3 times per week on a cycle ergometer supervised at a moderate intensity. Following the training, PON1 activity increased (~20%) significantly in individuals with
MetS without observed changes in HDLc, but the changes in PON1 were still lower than those without MetS.

Rector et al. [235] recruited 25 sedentary and overweight to obese individuals with MetS to evaluate oxidative stress following exercise training and weight loss. The exercise training lasted for 4 to 7 (average 6 months) months to induce a 10% weight loss. The participants were asked to restrict their caloric intake by approximately 500 kcals per day and participate in supervised exercise sessions of 45 minutes for 5 days per week at an intensity of 60% $\text{VO}_{2\text{max}}$. The exercise session elicited a 1500 to 2000 caloric expenditure per week. PON1 activity was unaltered with diet- and exercise-induced weight loss in obese and overweight individuals.

Table 2 - Summary of PON1 activity to training.

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Subjects</th>
<th>Exercise Protocol</th>
<th>Results</th>
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<tbody>
<tr>
<td>Tomas et al. [94]. Evaluate PON1 activity, lipid levels, and oxidized LDL concentration in 17 healthy young volunteers before and after a 16-weeks aerobic exercise training period.</td>
<td>Sedentary adult men and women</td>
<td>16 weeks, 5 days per week for 50 minutes by swimming, running, or aerobic games</td>
<td>PON1 Activity before training, 258 ± 135 U·L$^{-1}$ PON1 Activity after training, 253 ± 109 U·L$^{-1}$ $p &gt; 0.05$ Intra-assay CV (0.70%) Inter-assay CV (0.76%)</td>
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<tr>
<td>Richter et al. [232]. To assess whether endurance exercise which is known to be cardioprotective could beneficially influence PON1</td>
<td>Adult men and women with increased cardiovascular risk and coronary artery disease</td>
<td>12 weeks, 3–5 days per week for 40 minutes at 70 – 80% maximum HR by walking or running</td>
<td>PON1 Activity before training, 133 ± 6 U·L$^{-1}$ PON1 Activity after training, 130 ± 5 U·L$^{-1}$ $p &gt; 0.05$ CV was NR</td>
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<tr>
<td>Study</td>
<td>Participants</td>
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<td>Exercise Details</td>
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<tr>
<td>Goldhammer et al. [231]</td>
<td>Adult men and women with known CAD</td>
<td>12 weeks</td>
<td>3 days per week, 70 – 85% maximum HR on a treadmill, stationary bike, arm bicycle, rowing machine, or a combination of these activities.</td>
</tr>
<tr>
<td>Casella-Filho et al. [234]</td>
<td>20 Subjects with MetS (10 males and 10 females)</td>
<td>12 weeks</td>
<td>3 days per week, on a cycle ergometer</td>
</tr>
<tr>
<td>Rector et al. [235]</td>
<td>21 female and 9 male subjects with MetS</td>
<td>4 to 7 months</td>
<td>5 days per week supervised on a cycle ergometer or elliptical at an intensity of 60% VO₂max.</td>
</tr>
</tbody>
</table>

Significance, p < 0.05*; PON1, Paraoxonase 1; CAD, Coronary Artery Disease; MetS, Metabolic Syndrome; CV, Coefficient of Variation, NR, Not Reported.

In summary, training studies that involved subject exercising at moderate-intensity from 12 to 16 weeks have produced inconsistent results. PON1 activity was increased 16 to 19% in two studies [231, 234], while there was no change in the other three [94, 232, 235]. All of the investigators used aerobic forms of exercise including cycle ergometer, elliptical, treadmill, or swimming to investigate PON1 activity following a 12 to 28 week training program. The training program consisted of 3 to 5 days per week.
of exercise at an intensity of 70 to 85% maximum heart rate. The participants in each of these 5 studies included men and women that were either sedentary, diagnosed MetS, or diagnosed CAD. All samples were collected following an overnight fast. Finally, blood samples were collected before the training study and immediately following the conclusion of the exercise program.

**Acute Studies and PON1**

At present there are 4 studies designed to measure PON1 activity in response to an acute bout of exercise [94, 95, 236, 237]. As with exercise training, PON1 responses to acute exercise have conflicting results. Men and women that were sedentary, sedentary with T2DM, or trained served as participants. The conflicting results could be a result of subject selection, exercise intervention, and/or the timing of blood sample collection (Table 3).

Otocka-Kmiecik et al. [95] investigated a single bout of maximal exercise on PON1 activity in 29 trained male athletes. The average maximal oxygen consumption for the male participants was 59.8 ± 11.4 mL·kg⁻¹·min⁻¹. The participants were engaged in aerobic physical activity for the previous 3 years. Each participant completed a maximal exercise test on a treadmill. The blood sampling was done before exercise, immediately post-exercise, and 2-hours post-exercise. One maximal bout of exercise significantly increased PON1 activity (436.04 ± 275.17 U·L⁻¹) immediately after as compared to before exercise (371.43 ± 244.88 U·L⁻¹); however, the activity returned back to baseline levels within 2 hours (388.89 ± 256.43 U·L⁻¹).

Iborra et al. [237] recruited men and women with and without T2DM to investigate PON1 following an acute bout of exercise on a cycle ergometer. Participants with T2DM and control that participated in this investigation had maximal oxygen consumptions of 21.5 ± 3.1 mL·kg⁻¹·min⁻¹ and 26.9 ± 6.4 mL·kg⁻¹·min⁻¹. The participants
were asked to cycle for 40 minutes at 70 to 80% \( \text{VO}_{2\text{peak}} \). The blood sample was collected before exercise, 40 minutes post-exercise, and 24 hours post-exercise. PON1 activity did not respond to the acute bout of exercise.

Benitéz et al. [236] included 11 male athletes were asked to perform a single session of running that lasted four hours. The maximal oxygen consumption data were not reported. The sample was collected before exercise and immediately post-exercise. They found PON1 activity was unchanged.

Tomas et al. [94] recruited 10 women and 7 men that were apparently healthy and sedentary to examine the effects of exercise training on acute changes in PON1 activity. The participants were asked to ride a cycle ergometer for 30 minutes at their aerobic power output. The aerobic power output was determined 1 week prior to the acute bout of exercise. The average aerobic power output for the group was significantly increased following the 16-weeks of training. Therefore, the maximal oxygen consumption significantly increased from baseline was 37.4 ± 7.7 mL·kg\(^{-1}\)·min\(^{-1}\) to 46.2 ± 11.0 mL·kg\(^{-1}\)·min\(^{-1}\) following the training. The training program was reported previously in the training section. The participant’s acute bout consisted of them completing their maximal aerobic output on a cycle ergometer. The blood sampling for the acute bout was done before exercise and immediately post-exercise, 30 minutes post-exercise, 1 hour post-exercise, 2 hours post-exercise, and 24 hours post-exercise. The same was done following the training intervention. Following training, an acute bout of exercise on a cycle ergometer resulted in a significant increase in PON1 activity immediately after exercise as compared to before exercise.
<table>
<thead>
<tr>
<th>Purpose</th>
<th>Exercise Protocol</th>
<th>Subjects</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otocha-Kmiecik et al. [95]. To evaluate the participation of plasma PON1 in antioxidant defense in response to a single bout of maximal exercise.</td>
<td>Maximal treadmill test &lt;br&gt; Caloric expenditure</td>
<td>32 male sportsmen</td>
<td>PV decreases did not exceed 5%. Did not correct for PV Shifts &lt;br&gt; PON1 Activity before acute bout, 371.43 ± 244.88 U·L⁻¹ &lt;br&gt; PON1 Activity after after acute bout, 436.04 ± 275.17 U·L⁻¹ &lt;br&gt; <em>p &lt; 0.05</em> &lt;br&gt; CV was not reported.</td>
</tr>
<tr>
<td>Tomas et al. [94]. Evaluate PON1 activity, lipid levels, and oxidized LDL concentration before and after a 16-weeks aerobic exercise training period.</td>
<td>30 minutes at maximal aerobic power on cycle ergometer before and after training &lt;br&gt; Caloric expenditure</td>
<td>17 Sedentary adult men and women</td>
<td>PV was NR &lt;br&gt; Significant increase following an acute bout of exercise after training. The author did not list results for PON1 Activity. They only had a figure to show data. &lt;br&gt; <em>p &lt; 0.05</em> &lt;br&gt; Intra-assay CV (0.70%) &lt;br&gt; Inter-assay CV (0.76%)</td>
</tr>
<tr>
<td>Iborra et al. [237]. Analyze the effect of aerobic exercise training and a single bout of exercise on plasma oxidative stress and on antioxidant defenses in type 2 diabetes mellitus and in healthy control subjects.</td>
<td>40 minutes at 70 – 80% VO₂ peak on a cycle ergometer &lt;br&gt; Caloric expenditure</td>
<td>Males and females with and without T2DM</td>
<td>PV was NR &lt;br&gt; T2DM Group, PON1 Activity before acute bout, 109 ± 38 U·L⁻¹ &lt;br&gt; PON1 Activity after after acute bout, 106 ± 49 U·L⁻¹ &lt;br&gt; <em>p &gt; 0.05</em> &lt;br&gt; Control Group, PON1 Activity before acute bout, 102 ± 26 U·L⁻¹ &lt;br&gt; PON1 Activity after after acute bout, 105 ± 26 U·L⁻¹ &lt;br&gt; <em>p &gt; 0.05</em> &lt;br&gt; Inter-assay CV (8.14%)</td>
</tr>
</tbody>
</table>
Benitíez et al. [236]. Evaluate the immediate effects of intense aerobic exercise on the composition and oxidizability of low-density lipoproteins (LDL) and high-density lipoproteins.

<table>
<thead>
<tr>
<th>4 hour run Caloric expenditure</th>
<th>11 male athletes</th>
<th>Corrected for PV shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PON1 Activity before acute bout, 157.7 ± 21.6 U·L⁻¹
PON1 Activity after the acute bout, 153.1 ± 27.2 U·L⁻¹
p > 0.05
CV was not reported

Significance, p < 0.05*; PON1, Paraoxonase 1; T2DM, Type 2 Diabetes Mellitus; PV, Plasma Volume; NR = not reported; CV, coefficient of variation.

In summary, a total of four studies were conducted to investigate PON1 activity responses to an acute bout of exercise. Two investigators found PON1 activity increased by 18% following an acute bout of exercise [94, 95]. The other two research groups found that PON1 activity decreased by 2 to 3% following an acute bout of exercise, but the difference was not significant [236, 237]. The participants that volunteered for these four studies were sedentary, trained, or diagnosed with T2DM. There were two acute bouts that involved a maximal test performed on either a cycle ergometer or a treadmill [94, 95]. The third investigator asked the participants to ride a cycle ergometer for 40 minutes at 70 to 80% VO₂ peak [237]. The last investigation included 11 trained male athletes that performed a 4-hour run [236]. The two investigators that reported a significant increase in PON1 utilized a higher intensity of exercise than the two investigators that did not find a difference. There was no consistency with timing of blood collection and samples were collected following an overnight fast. Furthermore, the differences in PON1 activity responses to acute exercise could be due to the subject characteristics and exercise interventions. Only two of the four investigators corrected for plasma volume shifts [95, 236] and none reported caloric expenditure [94, 95, 236, 237]. The physical characteristics of the participants may provide a potential rationale for the increases in PON1. Second, the intensity of the
exercise could explain the increases in PON1 activity. Finally, supervised exercise training may provide an explanation for the changes in PON1.

**Myeloperoxidase and Exercise**

Myeloperoxidase (MPO) is a heme protein expressed in neutrophils that has been linked to cardiovascular disease [238] and plays an important role in the development of coronary artery disease [239]. Baldus et al. [240] found that MPO levels above the threshold of 350 ng·mL⁻¹ can predict adverse cardiac events in a normal population. Acute exercise causes local inflammation of the soft tissue, which results in a normal recruitment of neutrophils [241]. Neutrophils play an important role in the immune response by assisting in soft tissue repair following inflammation or injury after acute exercise [241]. The neutrophil response to damaged or inflamed tissue results in degranulation and release of MPO from the neutrophils [241]. Acute exercise has been shown to induce neutrophil degranulation that leads to an increase in concentration of plasma MPO levels [242-244] and the degranulation tends to increase with intensity of exercise [245].

Acute exercise in the animal model, Morozov et al. [242] utilized adult male rats and divided them into a trained and untrained group. The rats completed 15 intermittent swimming bouts with the addition of weight (8% of total body mass) for a total exercise time of 40 minutes. The trained group of rats had a higher resting plasma MPO level (0.98 µg·mL⁻¹) as compared to the untrained group (0.70 µg·mL⁻¹). The post-exercise plasma MPO levels significantly increased as compared to rest in both the trained and untrained (1.77 µg·mL⁻¹ and 1.66 µg·mL⁻¹), respectively. They concluded the higher resting plasma MPO levels in the trained group could be attributed to the adaptive response to intense training and results in a faster recovery from post-exercise inflammation.
Melanson et al. [243] recruited 24 amateur runners to compete in the Boston Marathon. Blood samples were drawn before and immediately after the marathon. The MPO increased from a mean of 281.44 pmol·L⁻¹ (42.2 ng·mL⁻¹) before the race to 785.21 pmol·L⁻¹ (117.8 ng·mL⁻¹) post race. The results were significant and they concluded that the elevation was due to an inflammatory response [243].

Niess et al. [246] recruited seven endurance trained athletes to participate in 60 minute treadmill runs at 75% \( \bar{\text{VO}}_{2\text{max}} \). The subjects ran in two conditions (18°C and 28°C) separated by 6 days to investigate how increased temperatures can affect the immune response. Blood samples were drawn before exercise, immediately post-exercise, 30 minutes post-exercise, 3 hours post-exercise, 24 hours post-exercise, and 48 hours post-exercise. The plasma MPO levels were more than 3 times higher immediately post-exercise as compared to pre-exercise levels for both conditions and the plasma MPO levels returned to pre-exercise levels within 3 hours of the exercise. They attributed the increase in plasma MPO levels to neutrophil activation due to muscle damage and heat stress does not seem to influence the acute immune response.

Wetzstein et al. [238] included 12 apparently healthy adults (5 sedentary, 7 active). This was a cross-sectional study to determine if an acute bout of exercise will induce oxidation of LDL and increase MPO levels. The subjects were asked to exercise on a treadmill for 30 minutes at 55% (sedentary) and 70% (exercise) of \( \bar{\text{VO}}_{2\text{peak}} \). The two groups were determined by the number of hours per week they exercised. The sedentary group exercised \( \leq 1 \text{ hr·wk}^{-1} \) and the exercise group exercise \( \geq 6 \text{ hr·wk}^{-1} \). The average \( \bar{\text{VO}}_{2\text{peak}} \) was 35.0 ± 4.5 mL/kg/min and 59.6 ± 8.3 mL/kg/min for the sedentary and exercise group, respectively. Blood samples were drawn prior to the exercise session and immediately post-exercise. The MPO level for the sedentary group (17.4 ng·mL⁻¹) was higher prior to exercise as compared to the exercise group (14.7 ng·mL⁻¹).
However, the author did not report significance between these two groups. The MPO levels of the sedentary group before exercise was 17.4 ng·mL⁻¹ and after exercise was 24.5 ng·mL⁻¹. The MPO levels of the exercise group before exercise was 14.7 ng·mL⁻¹ and after exercise was 18.2 ng·mL⁻¹. The results for both groups were not significant, which could be attributed to the low sample size. The MPO levels of the combined groups before exercise was 15.8 ng·mL⁻¹ and post-exercise was 20.8 ng·mL⁻¹. They found a significant increase in MPO following the acute bout of exercise when the investigators combined groups.

Camus et al. [247] investigated the effects of uphill walking and downhill running for 20 minutes at similar energy costs. The walking and running speeds were set according to an energy cost that was equivalent to 60% $\dot{VO}_{2\text{max}}$. They recruited 10 healthy males to walk up a 5% grade on a treadmill and ten days later the same participants came back to run downhill using a -20% grade to investigate neutrophil activation by measuring MPO. Blood samples were drawn before, immediately post exercise, and 20 minutes post exercise. The pre-exercise results for MPO was approximately 300 ng·mL⁻¹ for the walking and running group. The walking group MPO concentration increased from approximately 300 ng·mL⁻¹ to 400 ng·mL⁻¹, which was not a significant increase following the exercise session. However, the running group’s MPO level increased from approximately 300 ng·mL⁻¹ to 600 ng·mL⁻¹, which was a significant increase following the exercise session. Myeloperoxidase concentration significantly increased immediately post-exercise following the downhill run, but not in the walking uphill at the same energy cost. The increase in MPO in the downhill run suggests that the eccentric component of exercise activates polymorphonuclear cells (PMN) and could be a result of the acute inflammatory response to greater muscle damage as compared to uphill running.
Peake et al. [245] recruited 10 well-trained male runners and triathletes. The purpose of the study was to investigate the effects of moderate-intensity and high-intensity exercise on neutrophil degranulation. The participant’s average \( \dot{V}O_{2\text{max}} \) was 61.0 ± 3 mL/kg/min. The participants were asked to run for 60 minutes on two separate occasions, one at 60% moderate-intensity and the other at 85% high-intensity \( \dot{V}O_{2\text{max}} \). The order of the two trials was counterbalanced between the participants. Blood samples were collected before exercise, immediately post-exercise, and 1 hour post-exercise. The MPO for the moderate-intensity session remained approximately 10 ng·mL\(^{-1}\) from pre-exercise as compared to immediately post-exercise. However, the MPO levels for the high-intensity was significantly increased from approximately 10 ng·mL\(^{-1}\) to 30 ng·mL\(^{-1}\). These results were taken to indicate that neutrophil degranulation occurs to a greater extent during exercise when exercise intensity is high.

### Table 4 – Summary of MPO to acute exercise.

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Subjects</th>
<th>Exercise Protocol</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanson et al. [243]. Determine MPO levels following exercise.</td>
<td>24 Amateur runners</td>
<td>Completed Boston Marathon</td>
<td>MPO before marathon 42.2 ng·mL(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MPO after marathon 117.8 ng·mL(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( p &lt; .0001^* )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inter-assay CV = 13%</td>
</tr>
<tr>
<td>Niess et al. [246]. To investigate immune response following exercise.</td>
<td>7 Endurance Athletes</td>
<td>60 minute treadmill runs at 75% ( \dot{V}O_{2\text{max}} )</td>
<td>Results estimated from table. MPO before exercise 6 ng·mL(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MPO after exercise 30 ng·mL(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( p &lt; 0.05^* )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CV was NR</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>MPO before exercise</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Wetzstein et al.</td>
<td>5 Sedentary, 7 active</td>
<td>The sedentary group exercised on a treadmill for 30 minutes at 55% and active group exercised at 70% (exercise) of $V_O^{\text{peak}}$.</td>
<td>15.8 ng·mL$^{-1}$</td>
</tr>
<tr>
<td>Camus et al.</td>
<td>10 Apparently healthy males</td>
<td>The walking uphill and running downhill for 20 minutes at 60% $V_O^{\text{max}}$ on a treadmill</td>
<td>Walking uphill</td>
</tr>
<tr>
<td>Peake et al.</td>
<td>10 well-trained male runners and triathletes</td>
<td>Running for 60 minutes at 60% and 85% $V_O^{\text{max}}$ on a treadmill</td>
<td>60% $V_O^{\text{max}}$. MPO did not change.</td>
</tr>
</tbody>
</table>

MPO, myeloperoxidase; Significance, $p < 0.05^*$; nPMN, polymorphonuclear neutrophil count; EL, elastase; CV, Coefficient of Variation; NR, Not Reported.

In summary, MPO concentrations significantly increased between 30% and 97% following an acute bout of exercise [238, 243, 245, 246]. The blood sampling was similar for all investigations. Exercise of higher intensity and longer duration induced
greater increases in MPO. There were two investigations in which no changes in MPO following exercise was reported. In both instances, the reported exercise intensity was 60% $\dot{V}O_2$max. Each of the studies utilized aerobic forms of exercise on treadmills or participated in a marathon. MPO increases are transient lasting only hours after exercise is complete.

**oLDL and Exercise**

Physical inactivity contributes to the accumulation of central fat and the development of oxidative stress [248, 249]. The production of free radicals as a result of increased oxidative stress can cause modifications to lipoproteins, in particular LDL, which may result in the formation of oLDL and development of atherosclerosis [250]. Exercise has many health benefits for improving overall health and reducing oLDL. In support of this, several investigators have demonstrated that chronic exercise significantly reduces serum oLDL levels (Table 5) [94, 235, 251].

*Chronic Exercise and oLDL*

Chronic exercise has been shown to significantly reduce serum oLDL levels. Vasankari et al. [251] investigated oLDL over a ten month exercise program with 104 sedentary men and women. The exercise mainly included walking with additional exercises coming from skiing, biking, and circuit resistant training. A 23% and 26% decrease in oLDL for men and women was reported.

Blache et al. [252] conducted a twenty-week training program which included 146 apparently healthy men and women and children between the ages of 16 and 65. The participants were asked to come in the laboratory to exercise three times per week on a cycle ergometer. The intensity and duration was increased every two weeks until they
could achieve 50 minutes at 75% $\dot{V}O_{2\text{max}}$ for the last six weeks of the twenty-week training program. They found no change in oLDL.

Tomas et al. [94] measured oLDL in 17 apparently healthy men and women before and after 16-weeks of aerobic exercise training. The exercise training was previously described in the chronic exercise and PON1 section. Blood was collected following an overnight fast. The blood time draws were before training and following the 16-weeks of training. The 16-weeks of training significantly reduced oLDL from 48.8 ± 16.2 U·L$^{-1}$ to 41.1 ± 10.6 U·L$^{-1}$ [94].

<table>
<thead>
<tr>
<th>Table 5 - Summary of oLDL to chronic exercise.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose</td>
</tr>
<tr>
<td>---------</td>
</tr>
</tbody>
</table>
| Vasankari et al. [251]. Examine the effects of acute prolonged exercise on-serum and LDL oxidation and antioxidant defences | 104 Sedentary Men and Women | 10 Month Aerobic Exercise, mainly walking | Men, oLDL before training, 36.1 µmol·L$^{-1}$  
Men, oLDL after training, 27.9 µmol·L$^{-1}$  
$p < 0.05$*  
Women, oLDL before training, 26.2 µmol·L$^{-1}$  
Women, oLDL after training, 19.4 µmol·L$^{-1}$  
$p < 0.05$*  
Intra-assay CV = 4.4%  
Inter-assay CV = 4.5% |
| Blache et al. [252]. Examine the effect of exercise training on in vitro LDL oxidation and free radical-induced hemolysis: the HERITAGE Family Study. | 146 Men and Women | 20 Week Training program | The values for oLDL was not reported, only listed in text as not significant before and after training.  
$p > 0.05$ |
| Tomas et al. [94]. oLDL concentration were determined an acute bout of exercise before and after a 16-weeks aerobic exercise training period. | Sedentary adult men and women | 16 weeks, 5 days per week for 50 minutes | oLDL before training, 48.8 U·L⁻¹; oLDL after training, 41.1U·L⁻¹; \( p < 0.043^* \); Intra-assay CV = 2.8%; Inter-assay CV = 10.7% |

Significance, \( p < 0.05^* \); oLDL, oxidized low-density lipoprotein; LDL, Low-Density Lipoprotein; NC, No Change; CV, Coefficient of Variation.

In summary, chronic exercise significantly reduced oLDL by 18 to 29% [94, 251, 253] and only one reported no change [254]. The exercise programs consisted of 4 to 10 months of training. The participants for each of the studies were asked to exercise from 3 to 5 days per week. The duration ranged from 30 to 50 minutes of exercise per session. Each of the training programs utilized aerobic forms of exercise. The researchers for each of the studies collected blood samples before the training and following the training to compare the changes in oLDL. The methods for determining oLDL was different among each of the studies, but the results indicated a decrease in the amount of oLDL following training.

**Acute Exercise and oLDL**

Acute exercise increases oxidative because of the rapid production of free radicals from the increased reactive oxygen species production via oxygen consumption during the acute exercise [255]. The antioxidants to oxidants would be decreased. This could potentially lead to oxidative modification of LDL. However, there are conflicting
results. An acute bout of exercise increases circulating levels of oLDL, decreases oLDL, and has no effect on oLDL (Table 6) [94, 238, 256-258].

Tomas et al. [94] measured oLDL in 17 apparently healthy men and women as previously described. Blood samples were collected before exercise, immediately post-exercise, 30 minutes post-exercise, 1 hour post-exercise, 2 hours post-exercise, and 24 hours post-exercise. The researchers found that an acute bout of exercise did not alter oLDL (from 48.8 U·L⁻¹ to 54.0 U·L⁻¹) before training. On the other hand, the oLDL was significantly increased (from 41.1 U·L⁻¹ to 50.0 U·L⁻¹) following an acute bout of exercise after 16-weeks of training as compared to baseline following training. The results indicate that the increase in oLDL following the 16-weeks could be due to lower starting baseline levels of oLDL and the higher workload the participants were able to achieve.

Vuorimaa and colleagues [256] examined the effects of 6-hours of walking on 2 consecutive days using well-trained men on oLDL with and without a carbohydrate load. In a randomized order, the equally divided participants would either be in the placebo group or carbohydrate group. The placebo would drink a sweetened placebo containing 66 mg·L⁻¹ of Hermesetas® during the exercise session. The carbohydrate group would perform the same 6-hour walk, but would drink 50 g·L⁻¹ of carbohydrate (saccharose). Each group would be asked to walk for 6-hours at 55% heart rate reserve (HRR) on day 1 and day 2. This would be repeated in a randomized order separated by 14 days. The LDL oxidation was measured by determining the level of LDL diene conjugation. This is the separation of LDL from the sample and examining the oxidation characteristics by a spectrophotometric method [259]. The blood sample was baseline (PRE), drawn immediately post-exercise (POST1), 24 hours post-exercise (POST2), and immediately after the day 2 exercise (POST 3). This was repeated following the 14 days. The researchers found that oLDL was significantly reduced by 17% between PRE and POST
1 for the placebo group only. The placebo and carbohydrate lead to significant reductions in oLDL by 25% and 21% when you compare PRE and POST 3.

Vasankari et al. [254] included 8 highly trained marathon runners in trial I and 22 marathon runners in trial II and the distance for each trial was 31km. The LDL oxidation was measured by determining the level of LDL diene conjugation for trial I (from 52.8 ± 3.0 μmol·L⁻¹ to 57.3 ± 2.1 μmol·L⁻¹) and for trial II (from 33.6 ± 3.0 μmol·L⁻¹ to 34.5 ± 2.0 μmol·L⁻¹). They did not see any changes in oLDL following the acute prolonged exercise for both trials.

Chang et al. [257] examined 10 sedentary individuals as a control group, 15 trained rugby players (TR), and 6 weekend warriors (WW) to determine LDL oxidation and activities of antioxidant enzymes following a rugby match. The WW were active and did not participate in a training program. The TR group played on a rugby team and had a training regimen that included practicing three times per week and weight training. The blood samples were drawn before and after a rugby match. They found that both the TR and WW groups had a significantly increased oLDL following the rugby game as compared to before the rugby match. They also found the WW group had a significantly higher oLDL as compared to the TR group. The findings for the WW group were thought to be due to increased oxidative stress that could be a result of the exercise protocols and the fitness levels of the subjects.

Wetzstein et al. [238] conducted a cross-sectional study that included 12 apparently healthy adults in the sedentary group (4 males, 8 females) and 11 apparently healthy adults in the exercise group (5 males, 6 females). The two groups were determined by the number of hours per week they exercised. The sedentary group exercised ≤ 1 hr·wk⁻¹ and the exercise group exercise ≥ 6 hr·wk⁻¹. The average \( \dot{V}O_2 \text{peak} \) was 35.0 ± 4.5 mL/kg/min and 59.6 ± 8.3 mL/kg/min for the sedentary and exercise group, respectively. The subjects were asked to exercise on a treadmill for 30 minutes at
55% (sedentary) and 70% (exercise) of \( \dot{V}O_{2\text{peak}} \). The blood samples were collected following an overnight fast before and immediately following the acute bout of exercise. The researchers did not find a difference in the oxidation of LDL following an acute bout of exercise, when you examine each group individually. However, when you combine the results from both groups, there were significant increases in the oxidation of LDL following an acute bout of exercise. The findings suggest that moderate-intensity exercise is sufficient to increase oxidized LDL.

In contrast, Tozzi-Ciancarelli et al. [258] observed 15 apparently healthy, sedentary males to investigate LDL susceptibility to oxidation following an acute bout of exercise. The participants completed a maximal graded exercise on a cycle ergometer to determine their \( \dot{V}O_{2\text{max}} \). The participants returned seven days later to ensure complete recovery from the maximal exercise test and to complete the acute bout of exercise. The duration and intensity of the acute exercise session was 30 minutes at 60% \( \dot{V}O_{2\text{max}} \) on a cycle ergometer. The blood was collected in a fasting state before, immediately post-exercise, and 24 hours post-exercise following the maximal test and the acute bout of exercise. The maximal test on the cycle ergometer led to a significant increase in oLDL. The acute bout of exercise also caused a significant increase in oLDL, but oLDL returned to baseline levels 24 hours post-exercise.
## Table 6 - Summary of oLDL to acute exercise.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Exercise Protocol</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomas et al. [94]. Examine PON1 activity, lipid levels, and oxidized LDL concentration were determined an acute bout of exercise before and after a 16-weeks aerobic exercise training period.</td>
<td>Sedentary men and women</td>
<td>30 minutes at maximal aerobic power on cycle ergometer before and after 16 weeks of training</td>
<td>oLDL before training resulted in NC. oLDL Significantly increased following the training. No results were included in text and graph. Intra-assay CV = 2.8% Inter-assay CV = 10.7%</td>
</tr>
</tbody>
</table>
| Vuorimaa et al. [256]. Examine the effects of a 2-day walk exercise on the serum concentration of circulating moderately oxidized LDL. | 10 Well-trained men | Completed two 6-hour walk at 55% HRR on 2 consecutive days | PRE and POST 1. oLDL, Placebo 28 µmol·L⁻¹ and 23 µmol·L⁻¹  
$p < 0.01^*$  
Carb load, NC $p > 0.05$  
PRE and POST 3. oLDL, Placebo 28 µmol·L⁻¹ and 21 µmol·L⁻¹  
$p < 0.001^*$  
Carb load, 26 µmol·L⁻¹ and 21 µmol·L⁻¹ $p = 0.039^*$  
Intra- and Inter-assay variation was < 5%. |
| Vasankari et al. [254]. Examine the acute effects of long-distance running on oxidation of lipids and antioxidant functions in LDL and serum. | Trial I 8 Marathon Runners  
Trial II 22 Marathon Runners | 31 km Marathon | Trial I  
Before marathon, 52.8 µmol·L⁻¹  
After marathon, 57.3 µmol·L⁻¹ $p > 0.05$ |
<table>
<thead>
<tr>
<th>Study Authors (Year)</th>
<th>Participants</th>
<th>Design</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang et al. [257]. Investigate LDL oxidation and activities of antioxidant enzymes in WW at rest and after a rugby game.</td>
<td>15 Professional Rugby Players (TR) and 6 Recreational Weekend Rugby Players (WW) and 10 male Controls</td>
<td>Rugby Match and blood samples were taken before and after game</td>
<td>Baseline, TR, 19.77 µmol·L⁻¹, WW, 24.66 µmol·L⁻¹ vs. control, 16.07 µmol·L⁻¹. p &lt; 0.05* After Rugby Game, TR, 19.68 µmol·L⁻¹ vs. WW, 22.78 µmol·L⁻¹ vs. control, 16.07 µmol·L⁻¹. p &gt; 0.05</td>
</tr>
<tr>
<td>Wetzstein et al. [238]. Examine the effect of an acute exercise bout on the susceptibility of isolated low density lipoprotein to in vitro oxidation.</td>
<td>12 Sedentary men and women. 11 Active men and women</td>
<td>Treadmill, 30 minutes at 55% (sedentary) and 70% (exercise) of VO₂ peak</td>
<td>Sedentary group, NC, p &gt; 0.05. Exercise Group, NC, p &gt; 0.05. Combined Group, A decrease in lag time is associated with increased oxidation of LDL in vitro 96.1 to 92.1 minutes. p &lt; 0.05*</td>
</tr>
<tr>
<td>Tozzi-Ciancarelli et al. [258]. To evaluate in sedentary male</td>
<td>15 Sedentary males</td>
<td>Cycle Ergometer, Maximal test and 30 minutes at</td>
<td>Significantly increased oLDL immediately post-exercise</td>
</tr>
</tbody>
</table>
subjects the effects of an acute bout of strenuous and moderate exercise on oxidative markers.

|subjects the effects of an acute bout of strenuous and moderate exercise on oxidative markers. | 60% VO₂ max separated by 7 days | following maximal test, p < 0.05*; NC, following submaximal test, p > 0.05. CV was NR |

*Significant at the p < 0.05; oLDL, oxidized low-density lipoprotein; TR, trained rugby players; WW, untrained rugby players; NC, no change; CV, Coefficient of Variation; NR, Not Reported.

There were 3 reports that acute exercise increased oLDL by approximately 5% [94, 257, 258]. One investigator found that oLDL significantly decreased by approximately 25% [256]. Two investigators found that acute exercise had no effect on oLDL [238, 254]. The studies used a treadmill [238], walking [256], rugby match [257], marathon [254], and cycle ergometer [94, 258] to perform the acute bout of exercise. The intensity of the exercises ranged from 55 to 70% VO₂peak. The duration of the acute bout of exercise ranged from 30 minutes to 6 hours on two consecutive days. The participants were sedentary, active but not trained, and well-trained individuals. The blood samples were collected by the investigators before and after the acute bout.

**Composition of Lipoprotein Density and Size**

Lipoproteins are a heterogeneous group of particles which can be differentiated based on density, composition, and size [260]. The density of LDL and HDL can be determined by ultracentrifugation. The density for LDL ranges from 1.019 to 1.060 g·mL⁻¹ and HDL density ranges from 1.063 to 1.210 g·mL⁻¹ [261]. The LDL is composed of Apo B100 and the reference lipid composition has a total calculated lipid composition of 775 phospholipids, 387 unesterified cholesterol, 650 cholesterol esters, and 850 TG molecules [260]. HDL is mainly composed of Apo A and the HDL₂ particle has a
reference lipid composition of 137 phospholipids (i.e., phosphatidylcholine, lysolecithin, sphingomyelin), 50 unesterified cholesterols, 90 cholesterol esters, and 19 TG molecules [262, 263]. The HDL₃ particle has a reference lipid composition of 51 phospholipids, 13 unesterified cholesterols, 32 cholesterol esters, and 9 TG molecules [262, 263]. Traditionally, the size of the LDL and HDL particles was determined by gradient gel electrophoresis [264-266]. However, nuclear magnetic resonance (NMR) spectroscopy provides a new means to quantify lipoproteins based on the amplitudes of spectral signals emitted by the lipoprotein subclass [267]. NMR has provided another means for LDL and HDL to be divided into subclasses based on particle diameter. LDL can be divided into: Intermediate-density lipoprotein (IDL) (23 to 27 nm), large LDL (21 to 23 nm), and small LDL (18 to 21 nm) [268]. Small, LDL particles can be further divided into medium, small LDL particles (19.8 to 21.2) and very, small LDL particles (18 to 19.8) [268]. HDL can be divided into subclasses based on particle diameter: large HDL particles (8.8 to 13.0 nm), medium HDL particles (8.2 to 8.8 nm), and small HDL particles (7.3 to 8.2 nm) [268, 269].

**Low-Density Lipoprotein Particle Size and Exercise**

Lipoprotein particle size can provide more information outside of the traditional lipid profile [270-272]. These analyses are expensive and are not routinely performed. However, they can be invaluable when trying to see if interventions have the ability to reduce markers of oxidative stress. Small LDL particles are less buoyant than large LDL particles and are more atherogenic [273]. LDLc has been widely used as a risk factor for CVD, but determining LDL particle size is a more accurate measure to determine CVD risk [274].

Halverstadt et al. [220] investigated the effect of a 24-week endurance training program that included 100 older men and women that were sedentary. The exercise
session consisted of 20 minutes of exercise at 50% \( \dot{V}O_2 \text{max} \) for the first 10 weeks and then 40 minutes of aerobic exercise at 70% \( \dot{V}O_2 \text{max} \) for the final 14 weeks. They found that total LDL particle concentration (from 1436 ± 42 to 1336 ± 26 nmol·L\(^{-1}\); \( p = 0.01^* \)), medium LDL particle (from 221 ± 12 to 195 ± 7 nmol·L\(^{-1}\); \( p = 0.004^* \)), and very, small LDL particle size (from 745 ± 43 to 642 ± 27 nmol·L\(^{-1}\); \( p = 0.02^* \)) significantly decreased following the 24-week exercise program. Even though total LDL particle concentration decreased, the large LDL particle concentration significantly increased following the exercise program. The HDL particle size increased significantly following the exercise program (from 9.0 ± 0.0 to 9.1 ± 0.0 nm; \( p = 0.04^* \)). 24-weeks of endurance training favorably modified lipoprotein particles by reducing the number of small, atherogenic LDL particles, while increasing HDL particles.

Kraus et al. [275] investigated the effect of exercise dose and intensity of exercise on lipoproteins. The researchers recruited 159 men and women that were sedentary, overweight or mildly obese, and had dyslipidemia. There were only 84 of the 159 subjects that completed the exercise program due to various reasons (non-compliance, excessive weight loss, and incomplete lipid data). The subjects were divided into three groups: low-amount moderate-intensity (LM), low-amount high-intensity (LH), and high-amount high-intensity (HH). The LM exercise prescription would be equivalent to walking 12 miles per week at 40 to 55% \( \dot{V}O_2 \text{peak} \). The LH exercise prescription would be equivalent to jogging 12 miles per week at an intensity of 65 to 80% \( \dot{V}O_2 \text{peak} \). The HH exercise prescription would be equivalent to jogging 20 miles per week at an intensity of 65 to 80% \( \dot{V}O_2 \text{peak} \). They found that all three forms of exercise (LM, LH, and HH) significantly increased the size of LDL particles as compared to control. Only the concentration of small LDL cholesterol, concentration of LDL particles, and concentrations of IDL cholesterol were significantly reduced in the HH group as
compared to control. Furthermore, HDL concentration, concentration of large HDL cholesterol, and HDL particle size were significantly increased only in the HH group as compared to control. They also were able to demonstrate that jogging for 17 to 18 miles increased HDL particle and concentration, while decreasing VLDL triglycerides and large VLDL particles [275]. The investigators concluded that it is the amount of exercise rather than fitness levels that are necessary for improvement in lipid profiles and reduced CVD risk.

Elevated LDL cholesterol has traditionally been related to the increased incidence and progression of CVD [1]. However, lipoprotein particle size analysis can provide the clinician with more detailed information about the overall risk of CVD [268]. It is the smaller, more, dense LDL particles which are more capable of penetrating the vascular endothelium, becoming oxidized and contributing more to the development of the atherosclerotic lesions. The antioxidant activities of HDL can largely be attributed to the activities of PON1. There is evidence that exercise can increase HDLc, HDL particle size, and reduce LDL particle size. There is limited evidence that exercise can increase the activity and concentration of PON1.

Increased lipoprotein lipase activity (LPL), as a result of exercise, appears to be the mechanism that causes a reduction in TG concentrations [82, 219]. However, the exact mechanism by which LPL activity alters particles size is still unclear. Increase LPL activity increases HDLc and increases the HDL particle size, which may explain greater HDL₂ subfractions after exercise [123, 220, 276]. Harrison et al. [277] proposed that a decrease secretion of large VLDL particles from the liver may provide evidence that skeletal muscle LPL may play an important role in the reduction of downstream formation of small, dense LDL particles. In particular, the decreases in TG content of the large VLDL particles resulted in a reduction in the large VLDL particles.
**Niacin**

Extended-release niacin is a pharmacologic drug that has been shown to significantly reduce TG, increase HDLc, and increase LDL particle size. The use of extended-release niacin as a therapy to ameliorate dyslipidemia may potentially reduce the risk of CVD and improve overall health [109, 278, 279]. The exact means by which niacin influences lipid metabolism is not known. However, research evidence suggest the mechanism for niacin appear to be influenced by reductions in adipocyte TG lipolysis, VLDL secretion, and inhibition of the removal of HDL by the liver.

Niacin is an essential B vitamin (B₃) that has lipid lowering effects when used in prescription doses [109, 278]. Niacin has been shown to be effective at improving dyslipidemia by reducing triglyceride concentrations by approximately 30% and increasing HDLc up by 25% [99-102, 278-280]. However, an increase in the concentration of TG-rich lipoproteins, such as chylomicrons and VLDL, allows for increased translocation of cholesterol esters from HDL to TG-rich lipoproteins in exchange for TG. This transfer results from the action of CETP and TG-rich HDL particles are hydrolyzed by hepatic lipase and rapidly cleared from the plasma [281]. A similar CETP transfer of TG from VLDL to LDL contributes to the formation of small, dense LDL particles [261]. This remodeling occurs primarily in the liver before VLDL enters the peripheral circulation [261]. The increased TG levels are the driving force for reduced HDLc levels [282]. Elam et al. [279] recruited participants to participant in the Arterial Disease Multiple Intervention Trial (ADMIT). Niacin was titrated from 50 to 3000 mg·day⁻¹. The researchers found that niacin significantly increase HDLc by 29% and decreased TG concentrations by 23% in individuals that were overweight and had T2DM and peripheral artery disease. In another study, Grundy and colleagues investigated the use 1500 mg/day of extended release niacin in obese T2DM and they found an increase in HDLc by 24% and a decrease in TG by 28% [278]. The favorable changes seen with
extended-release niacin on HDLc and TG are also seen with favorable changes with
particle size distribution of HDL and LDL. Kuvin et al. [283] investigation included 50
men and 4 women to determine the effects of a 12-week therapy of niacin on lipoprotein
particle size. The participants were equally divided into a placebo group and niacin
group. The participants were asked to take one 500 mg tablet of extended-release
niacin for the first two weeks and then two tablets a day (1000 mg) for the remainder of
the study. Niacin therapy resulted in a 32% increase in large HDL particles and an 8%
decrease in small HDL particles. Additionally, niacin reduced the number of small LDL
particles by 12% and increased the large LDL particles by 82%.

The mechanism of action for niacin in the regulation of lipid metabolism is not
fully understood. Ganji et al. [284] reported that niacin directly and indirectly inhibited
hepatic diacylglycerol acyltransferase-1 (DGAT2), an enzyme that mediates the
conversion of VLDL to LDL and decreases the synthesis of TG. Adipose cells are
specialized for the synthesis and storage of TG and for their mobilization of FFA.
Carlson et al. [285] demonstrated that niacin decreased the release of FFA from adipose
tissue by inhibiting TG lipolysis. The activation of PPAR-γ by niacin could explain the
decreased release of FFA from adipose tissue. Activating PPAR-γ increases lipid
uptake by adipocytes and may act directly on the muscle to utilized energy produced
from fat stores [286]. PPAR-γ also increases LPL activity to remove excess circulating
levels of TG [287].

Recently, the identification of G protein-coupled receptor (GPR) 109A has
provided new insight to the action of niacin on adipocytes and immune cells [288]. In the
adipocytes, the beneficial effects of niacin to inhibit TG lipolysis is mediated by GPR
109A [289]. The unwanted side effects of flushing caused by niacin is regulated through
GPR 109A receptors on Langerhans cell in the skin [289]. Niacin also increases HDL
concentration by decreasing the fractional catabolic rate of HDL and Apo A1 in the liver
The decreased clearance of HDL would allow HDL size to increase from HDL₃ to HDL₂ and enhance reverse cholesterol transport [290]. Currently, there are not any investigations on the effects of niacin on PON1, so therefore this investigation may provide novel additional information to the beneficial effects of niacin.

**Summary and Conclusions**

Exercise and niacin therapy have been prescribed to reduce health risks associated with CVD. Physical inactivity, poor diet, accumulation of body fat, and IR may all cluster together to contribute to dyslipidemia and increase cardiovascular and metabolic risk. Cross-sectional studies suggest that exercise increases PON1, but really have limited information because PON1 have only been studied in adolescents in sports. Researchers have reported varying results on the response of PON1 to training. Three of the five research groups found no change in PON1, while the other two found a significant increase. One of the training studies also included an acute bout of exercise prior to training and immediately following training. This group found that an acute bout increased PON1 following training, but an acute bout did not change PON1 prior to training. Never the less, there is some evidence that suggests exercise increases PON1. There is limited evidence on the effect of niacin on PON1. However, with respect to PON1 regulation, there is sufficient evidence in cell cultures, animal models, and human models that niacin may up-regulate PPAR-γ and this may be a modulator of PON1 expression. Both exercise and niacin as therapeutic agents may reduce inflammation and improve antioxidant status of PON1. Even though exercise and niacin improve dyslipidemia by different mechanisms there is relatively little information on the mechanisms to increase PON1. To our knowledge, there is strong evidence that these two interventions may work in concordance with each other and to our knowledge this is the first investigation to demonstrate PON1 changes in adult males with MetS. So taken
together, exercise and niacin therapy may work together to increase PON1 activity and concentration, which may improve the antioxidant potential, reduce oLDL, and potentially contribute to the reduction of CVD risk.
CHAPTER III.
METHODOLOGY

General Overview

Twelve obese male participants, meeting the NCEP-ATP III criteria for MetS, completed a single session of exercise on a treadmill at 60 to 70% of $\dot{V}O_{2\text{max}}$ to expend 500 kcals. Participants who met the following criteria were included in the study: sedentary, non-smoking males between the ages of 30 and 65, obese (BMI $\geq$ 30 kg·m$^{-2}$), hypertriglyeridemic (TG $\geq$ 150 mg·dL$^{-1}$) and no contraindications to aspirin and niacin therapy. A fasting blood sample was obtained prior to exercise and 24 hours after exercise. Next, extended-release niacin was physician-prescribed and participants completed 6 weeks of niacin therapy. Extended release niacin was titrated by 500 mg·day$^{-1}$ during the first week to 1500 mg·day$^{-1}$ during the third week. The 1500 mg·day$^{-1}$ dose was maintained from the third week through the sixth week. Following the niacin intervention, all participants returned to complete exercise and blood sampling as before. Diet and physical activity records were self-reported during the blood sampling period [98].

The original study, approved by Auburn University Institutional Review Board, compared the independent and combined effects of acute exercise and 6-weeks of niacin therapy on post-prandial triglyceride responses in men with MetS. The methods used to collect the human participant data are published elsewhere [98]. The purpose of this investigation was to determine the independent and combined effects of exercise and extended release niacin on the concentration and activity of PON1 in men with MetS.
Exercise

Participants completed laboratory-based treadmill walking one week following a maximal graded exercise to expend 500 kcals at 60 to 70% VO2max. The caloric expenditure was estimated by measuring expired gas (oxygen and carbon dioxide) fractions sampled at the mouth using a pneumotach, and breath by breath gas analysis system (Medical Graphics, St. Paul, MN).

Extended-Release Niacin Administration

Participants were prescribed extended-release niacin by a physician. The extended-release niacin was titrated from 500 to 1500 mg·day⁻¹ over a three week period. Participants were asked to begin taking 500 mg·day⁻¹ right before bed for the first week. Dosage increased to 1000 mg·day⁻¹ for the second week. Dosage was increased to 1500 mg·day⁻¹ from week 3 to the end of the study (week 6). Participants were asked to take a 300 mg aspirin per day along with the niacin to reduce the risk of flushing [98]. There were only two of the original 15 participants that reported more than one episode of flushing due to the niacin therapy.

Biochemical Analysis

PON1 Concentration: Enzyme linked immunosorbent assay (ELISA) was used to determine the PON1 concentration in serum. The ELISA utilizes antibodies specific to PON1, with no cross-reactivity with PON2 and PON3. PON1 was determined using commercially available ELISA kit (Catalog# E0243HU, USCN Life Sciences Inc.; Wuhan, China). The samples and standards were analyzed in duplicate. All of the samples, excluding the standards, were diluted 1:1000 with 0.02 M phosphate buffered saline (PBS) that is not supplied in the kit. PBS diluted serum (100µL) was added to each of the 96 wells coated with anti-PON1. The plate was incubated for 2 hours covered at
37°C. Once the incubation period is over then 100μL of a detection reagent was added followed by an incubation of 1 hour covered at 37°C. A 96 well plate washer (Biotek Instruments Inc.; Winooski, VT) was used to wash the plate 3 times following the incubation and tapped dry on a paper towel. Once the incubation period is over then 100μL of a second detection reagent was added followed by an incubation of 30 minutes covered at 37°C. The wash procedure will follow the same steps as above. There was 90μL of substrate added to each well and incubated for 20 minutes covered at 37°C to allow for blue color development. Finally, 50μL of stop solution was added to each well to stop the reaction and change the color from blue to yellow. The plate will then be placed onto a 96 well plate reader (Biotek Instruments Inc.; Winooski, VT) at 450nm. The concentration for each specimen was calculated based on a four-parameter logistic curve fit. The four-parameter logistic fit will compare the unknown absorbances with known standard values to determine the concentration for the unknown samples. Finally, each unknown concentration will then be multiplied by the dilution factor of 1000 to determine the final concentration.

PON1 Activity: This blood marker is a primary variable of interest and was analyzed to determine the activity of PON1. The activity of PON1 is measured using arylesterase reagents and PON2 and PON3 do not have this activity using arylesterase reagent [291-293]. PON1 activity was determined by using a commercially available enzymatic kit (Catalog# 0801199, Zeptometrix Corporation; Buffalo, NY). The samples were analyzed in duplicate. The working reagent was prepared by adding 25μL of arylesterase substrate to 50 mL of assay buffer. The samples and standards were diluted 1:3 with arylesterase assay buffer. The spectrophotometer was turned on and set to a wavelength of 270nm. Water was used to zero the spectrophotometer before each run of standard and sample. A small volume of 6.67μL of sample and standard
was added to 1mL of working reagent in a single cuvette. This cuvette was inverted 3
times to allow for adequate mixing of the reagent and sample. A timer was started and
at 20 seconds the initial absorbance was recorded and then the final absorbance is
recorded at 80 seconds while in the spectrophotometer under a stable and constant
temperature. The following calculation was used to determine the activity of PON1:

\[
PON1 \text{ Activity} = [(\text{Final Absorbance} - \text{Initial Absorbance}) - \text{Blank}] \times 115
\]

Apo A1 Concentration: Apo A1 was analyzed because it is a structural
component of HDL and are required for the selective uptake of esterified cholesterol by
the liver [294]. Apo A1 was determined using commercially available ELISA kit
(Catalog# EA5201-1, AssayPro LLC.; St. Charles, MO). The samples and standards
were analyzed in duplicate. All of the samples, excluding the standards, were diluted
1:800 with diluents supplied by the manufacturer. First, 25µL of sample was added to
each of the 96 wells coated with anti-Apo A1 followed immediately with 25µL of
biotinylated Apo A1. The microtiter plates were incubated at room temperature for 2
hours. A 96 well plate washer (Biotek Instruments Inc.; Winooski, VT) was used to wash
the plate 4-5 times following the incubation and tapped dry on a paper towel. Next, 50µL
of streptavidin conjugate was added to each well and incubated for 30 minutes at room
temperature. The wash cycle was repeated as listed above. The addition of 50µL of
chromogen substrate was added to each well and incubated for 10 minutes to allow for
blue color development. Finally, 50µL of stop solution was added to each well to stop
the reaction and change the color from blue to yellow. The plate will then be placed onto
a 96 well plate reader (Biotek Instruments Inc.; Winooski, VT) at 450nm. The
concentration for each specimen was calculated based on a four-parameter logistic
curve fit. The four-parameter logistic fit will compare the unknown absorbances with
known standard values to determine the concentration for the unknown samples. Each concentration will then be multiplied by the dilution factor of 800.

Oxidized Low Density Lipoprotein Concentration: Oxidized Low Density Lipoprotein (oLDL) is a marker of oxidative stress and was analyzed primarily to determine the concentration of oLDL in serum. The concentration of oLDL was determined using commercially available ELISA kit (Catalog# BI20042, ALPCO Diagnostics; Salem, NH). The samples and standards were analyzed in duplicate. All of the samples, excluding the standards, were diluted 1:10 with assay buffer that is supplied by the manufacturer. First, 100µL of sample was added to each of the 96 wells coated with anti-oLDL and incubated at 37°C for 2 hours. A 96 well plate washer (Biotek Instruments Inc.; Winooski, VT) was used to wash the plate 5 times following the incubation and tapped dry on a paper towel. Next, 100µL of conjugate was added to each well and incubated for 1 hour at 37°C. The wash cycle was repeated as listed above. Next, 100µL of chromogen substrate was added to each well and incubated for 30 minutes in the dark to allow for blue color development. Finally, 50µL of stop solution was added to each well to stop the reaction and change the color from blue to yellow. The plate will then be placed onto a 96 well plate reader (Biotek Instruments Inc.; Winooski, VT) at 450nm. The concentration for each specimen was calculated based on a four-parameter logistic curve fit. The four-parameter logistic fit will compare the unknown absorbances with known standard values to determine the concentration for the unknown samples. Each concentration will then be multiplied by the dilution factor of 10.

Myeloperoxidase Concentration: Myeloperoxidase (MPO) is a heme protein that is released from macrophages and/or neutrophils that respond to inflammation [22].
Individuals with coronary artery disease have increased levels of MPO [239]. The concentration of myeloperoxidase (MPO) was determined using commercially available ELISA kit (Catalog# K6631A, ALPCO Diagnostics; Salem, NH). The samples and standards were analyzed in duplicate. All of the samples, excluding the standards, were diluted 1:40 with sample buffer that is supplied by the manufacturer. First, 100µL of sample was added to each of the 96 wells coated with anti-MPO and incubated at room temperature on a horizontal mixer for 1 hour. A 96 well plate washer (Biotek Instruments Inc.; Winooski, VT) was used to wash the plate 5 times following the incubation and tapped dry on a paper towel. Add 100 µL of detection antibody to each well and incubate for 1 hour at room temperature covered. The wash procedure is repeated as listed above. Next, 100µL of streptavidin conjugate was added to each well and incubated for 1 hour at room temperature covered. The wash cycle was repeated as listed above. Next, 100µL of chromogen substrate was added to each well and incubated for 15 minutes to allow for blue color development. Finally, 50µL of stop solution was added to each well to stop the reaction and change the color from blue to yellow. The plate will then be placed onto a 96 well plate reader (Biotek Instruments Inc.; Winooski, VT) at 450nm. The concentration for each specimen was calculated based on a four-parameter logistic curve fit. The four-parameter logistic fit will compare the unknown absorbances with known standard values to determine the concentration for the unknown samples. Each concentration will then be multiplied by the dilution factor of 40.

Lipoprotein Particle Size: Blood was collected in a red top tube and allowed to clot. The blood was centrifuged at 3000 g for 15 minutes to separate the serum from the cells. The serum was transferred to a plastic tube and frozen. The frozen serum samples were sent to Liposcience Inc. for nuclear magnetic resonance (NMR) testing.
NMR allows for determination of lipoprotein subclass particle size and number. The serum sample is exposed to a short pulse of radio energy within a strong magnetic field. The resonant sound that is made by the lipoproteins in the sample is recorded and analyzed to determine the number and size of lipoproteins present. Each type of lipoprotein makes a signal that is distinctly unique from the other lipoproteins. A computer algorithm is used to place the signals into groups and then quantifies the number of lipoprotein particles in that group [267, 269]. VLDL, LDL, and HDL particle numbers and sizes were determined by NMR (Liposcience Inc.; Raleigh, NC).

The normal ranges for large VLDL particle numbers, medium VLDL particle numbers, and small VLDL particle numbers are 0.1 – 8.5 nmol·L⁻¹, 4.3 – 114.9 nmol·L⁻¹, and 1.4 – 61.6 nmol·L⁻¹. The normal ranges for total LDL particle numbers, intermediate LDL particle numbers, large LDL particle numbers, small LDL particle numbers, medium small LDL particle numbers, and very small LDL particle numbers are 972 – 2195 nmol·L⁻¹, 0 – 86 nmol·L⁻¹, 70 – 657 nmol·L⁻¹, 516 – 1886 nmol·L⁻¹, 119 – 402 nmol·L⁻¹, and 393 – 1483 nmol·L⁻¹. The ranges for total HDL particle numbers, large HDL particle numbers, medium HDL particle numbers, and small HDL particle numbers are 19.9 – 36.5 µmol·L⁻¹, 1.6 – 10.1 µmol·L⁻¹, 0 – 6.8 µmol·L⁻¹, and 14.0 – 26.9 µmol·L⁻¹. The ranges VLDL particle size, LDL particle size, and HDL particle size are 41.1 – 65.9 nm, 19.5 – 21.5 nm, and 8.3 – 9.3 nm [268].

**Statistical Analysis**

Group means and standard deviations were determined for the following descriptive statistics: age (yrs); height (cm); weight (kg); BMI (kg·m⁻²); WHR; $\dot{V}O_2_{max}$ (mL·kg⁻¹·min⁻¹). The dependent variables of interest are PON1 concentration, PON1 activity, MPO, Apo A1, oLDL, and lipoprotein particle size and number for HDL and LDL. The Wilkes test for normality was used to determine if the baseline variables of interests
were normally distributed. A multiple 1 group (group) x 4 (sampling point) repeated measures ANOVA was used to determine significant changes in variables of interest. A comparison wise alpha level was set at $p < 0.05$. Duncans NMR tests were used to follow up the global tests. Pearson Product Moment correlations were determined to characterize relationships between baseline concentrations and changes in variables of interest.
CHAPTER IV.
RESULTS

Participants

The baseline physiological characteristics are provided in Table 7a. The participant’s baseline variables of interest are in Table 7b. All participants maintained their body weight throughout the study [98].

Table 7a – Baseline physiological characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Mean ± SE</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>yrs</td>
<td>44 ± 2</td>
<td>37</td>
<td>51</td>
</tr>
<tr>
<td>Height</td>
<td>cm</td>
<td>175.6 ± 2.8</td>
<td>165.5</td>
<td>185.7</td>
</tr>
<tr>
<td>Body Weight</td>
<td>kg</td>
<td>106.9 ± 5.1</td>
<td>88.4</td>
<td>125.4</td>
</tr>
<tr>
<td>BMI</td>
<td>kg/m²</td>
<td>34.5 ± 0.9</td>
<td>31.1</td>
<td>37.9</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>cm</td>
<td>108.8 ± 8.2</td>
<td>100.6</td>
<td>117.0</td>
</tr>
<tr>
<td>% fat</td>
<td>% of body weight</td>
<td>35 ± 1</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>VO₂max</td>
<td>mL·min⁻¹·kg⁻¹</td>
<td>27.5 ± 1.6</td>
<td>21.9</td>
<td>33.1</td>
</tr>
<tr>
<td>Glucose</td>
<td>mg·dL⁻¹</td>
<td>108 ± 5</td>
<td>69</td>
<td>147</td>
</tr>
<tr>
<td>Insulin</td>
<td>mU·mL⁻¹</td>
<td>17.5 ± 2.5</td>
<td>8.3</td>
<td>26.7</td>
</tr>
<tr>
<td>G/I ratio</td>
<td></td>
<td>7.9 ± 1.6</td>
<td>2.4</td>
<td>13.4</td>
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<tr>
<td>HOMA score</td>
<td></td>
<td>4.4 ± 0.6</td>
<td>2.4</td>
<td>6.4</td>
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<tr>
<td>NEFA</td>
<td>mmol·L⁻¹</td>
<td>0.49 ± 0.03</td>
<td>0.39</td>
<td>0.59</td>
</tr>
<tr>
<td>HDL-C</td>
<td>mg·dL⁻¹</td>
<td>41 ± 2</td>
<td>27</td>
<td>52</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>mg·dL⁻¹</td>
<td>293 ± 28</td>
<td>179</td>
<td>453</td>
</tr>
</tbody>
</table>

Values are presented as means ± standard error along with minimum and maximum values in range. NEFA = non-esterified fatty acids; HOMA score is homeostasis model score; G/I ratio = glucose to insulin ratio. Table modified from Plaisance et al. [98].
Table 7b – Baseline variables of interest.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Mean ± SE</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1a</td>
<td>kU·L⁻¹</td>
<td>125.9 ± 4.9</td>
<td>109.0</td>
<td>142.8</td>
</tr>
<tr>
<td>PON1c</td>
<td>µg·mL⁻¹</td>
<td>112.4 ± 8.2</td>
<td>84.0</td>
<td>140.8</td>
</tr>
<tr>
<td>Apo A1</td>
<td>µg·mL⁻¹</td>
<td>5031.5 ± 1646.1</td>
<td>&lt;0.5</td>
<td>10733.8</td>
</tr>
<tr>
<td>MPO</td>
<td>ng·mL⁻¹</td>
<td>718.7 ± 92.1</td>
<td>399.6</td>
<td>1037.8</td>
</tr>
<tr>
<td>oLDL</td>
<td>ng·mL⁻¹</td>
<td>1476.2 ± 699.1</td>
<td>&lt;0.5</td>
<td>3898.0</td>
</tr>
<tr>
<td>Large VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>5.7 ± 1.0</td>
<td>0.5</td>
<td>13.3</td>
</tr>
<tr>
<td>Medium VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>31.5 ± 5.3</td>
<td>10.4</td>
<td>74.5</td>
</tr>
<tr>
<td>Small VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>48.6 ± 3.8</td>
<td>29.2</td>
<td>71.0</td>
</tr>
<tr>
<td>Total LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1788.3 ± 105.1</td>
<td>1424.3</td>
<td>2152.3</td>
</tr>
<tr>
<td>Intermediate LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>43.6 ± 13.1</td>
<td>0</td>
<td>88.9</td>
</tr>
<tr>
<td>Large LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>264.7 ± 47.5</td>
<td>31.0</td>
<td>600.0</td>
</tr>
<tr>
<td>Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1480.0 ± 111.2</td>
<td>878.0</td>
<td>1978.0</td>
</tr>
<tr>
<td>Medium Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>312.4 ± 25.0</td>
<td>162.0</td>
<td>437.0</td>
</tr>
<tr>
<td>Very Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1167.6 ± 86.9</td>
<td>717.0</td>
<td>1541.0</td>
</tr>
<tr>
<td>Total HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>31.5 ± 1.3</td>
<td>26.9</td>
<td>36.1</td>
</tr>
<tr>
<td>Large HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>3.5 ± 0.5</td>
<td>1.8</td>
<td>5.3</td>
</tr>
<tr>
<td>Medium HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>5.1 ± 1.5</td>
<td>0</td>
<td>10.2</td>
</tr>
<tr>
<td>Small HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>22.9 ± 1.4</td>
<td>18.0</td>
<td>27.8</td>
</tr>
<tr>
<td>VLDL Particle Size</td>
<td>nm</td>
<td>58.4 ± 2.4</td>
<td>40.6</td>
<td>71.2</td>
</tr>
<tr>
<td>LDL Particle Size</td>
<td>nm</td>
<td>20.1 ± 0.1</td>
<td>19.4</td>
<td>21.1</td>
</tr>
<tr>
<td>HDL Particle Size</td>
<td>nm</td>
<td>8.6 ± 0.1</td>
<td>8.2</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Values are presented as means ± standard error along with minimum and maximum values in range. PON1a, paraoxonase 1 activity; PON1c, paraoxonase 1 concentration; Apo A1, Apolipoprotein A1; MPO, myeloperoxidase concentration; oLDL, oxidized low density lipoprotein concentration; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein.

Paraoxonase1 Response to Exercise

The independent effects of exercise were characterized with a single exercise session prior to initiating niacin therapy by comparing baseline measures with 24-hours post-exercise measures. The mean ± standard error for PON1 activity, PON1
concentration, Apo A1, HDL particle numbers, HDL particle size, HDLc, and TG are presented in Table 8.

**Table 8 – Response to exercise.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Baseline</th>
<th>24 Post Exercise</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1a</td>
<td>kU·L⁻¹</td>
<td>125.9 ± 4.9</td>
<td>131.5 ± 4.0</td>
<td>0.269</td>
</tr>
<tr>
<td>PON1c</td>
<td>µg·mL⁻¹</td>
<td>112.4 ± 8.2</td>
<td>118.9 ± 8.5</td>
<td>0.391</td>
</tr>
<tr>
<td>Apo A1</td>
<td>µg·mL⁻¹</td>
<td>5031.5 ± 1646.1</td>
<td>5052.7 ± 1325.6</td>
<td>0.983</td>
</tr>
<tr>
<td>Total HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>31.5 ± 1.3</td>
<td>32.1 ± 1.7</td>
<td>0.469</td>
</tr>
<tr>
<td>Large HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>3.5 ± 0.5</td>
<td>3.1 ± 0.5</td>
<td>0.068</td>
</tr>
<tr>
<td>Medium HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>5.1 ± 1.5</td>
<td>4.2 ± 1.3</td>
<td>0.496</td>
</tr>
<tr>
<td>Small HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>22.9 ± 1.4</td>
<td>24.9 ± 1.5</td>
<td>0.214</td>
</tr>
<tr>
<td>HDL Particle Size</td>
<td>nm</td>
<td>8.6 ± 0.1</td>
<td>8.5 ± 0.1</td>
<td>0.040*</td>
</tr>
<tr>
<td>HDLc</td>
<td>mg·dL⁻¹</td>
<td>41 ± 2</td>
<td>40 ± 2</td>
<td>0.403</td>
</tr>
<tr>
<td>TG</td>
<td>mg·dL⁻¹</td>
<td>293 ± 28</td>
<td>231 ± 28</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

All values are means ± SEM. * = Significant difference between conditions (p < 0.05). Baseline, Before exercise (Control); 24 hours post-exercise; PON1a, Paraoxonase 1 Activity; PON1c, Paraoxonase 1 Concentration; Apo A1, Apolipoprotein A1; HDL, High-Density Lipoprotein; HDLc, High-Density Lipoprotein cholesterol; TG, Triglyceride concentration.

Exercise did not alter PON1 activity (F₁,₁₁ = 1.36, p = 0.269) or PON1 concentration (F₁,₁₁ = 0.80, p = 0.391). Apo A1 was not altered (F₁,₁₁ = 0.00, p = 0.983).

Exercise did not influence a change in the total HDL particle numbers (F₁,₁₁ = 0.56, p = 0.469), large HDL particle numbers (F₁,₁₁ = 4.11, p = 0.068), medium HDL particle numbers (F₁,₁₁ = 0.50, p = 0.496), and small HDL particle numbers (F₁,₁₁ = 1.74, p = 0.214). However, the HDL particle size was reduced significantly (F₁,₁₁ = 5.39, p = 0.040*). HDLc was not altered (F₁,₁₁ = 0.758, p = 0.403), but triglyceride was significantly decreased (F₁,₁₁ = 11.40, p = 0.006*) following an acute bout of exercise.
The means + standard error for VLDL particle numbers, VLDL particle size, LDL particle numbers, LDL particle size, and oLDL responses to acute exercise are presented in Table 9.

**Table 9 – VLDL & LDL response to exercise.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Baseline</th>
<th>24 Post Exercise</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>5.7 ± 1.0</td>
<td>5.5 ± 1.0</td>
<td>0.321</td>
</tr>
<tr>
<td>Medium VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>31.5 ± 5.3</td>
<td>28.2 ± 3.9</td>
<td>0.222</td>
</tr>
<tr>
<td>Small VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>48.6 ± 3.8</td>
<td>56.0 ± 4.9</td>
<td>0.016*</td>
</tr>
<tr>
<td>VLDL Particle Size</td>
<td>nm</td>
<td>58.4 ± 2.4</td>
<td>56.4 ± 2.1</td>
<td>0.102</td>
</tr>
<tr>
<td>Total LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1788.3 ± 105.1</td>
<td>1829.2 ± 111.3</td>
<td>0.272</td>
</tr>
<tr>
<td>Intermediate LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>43.6 ± 13.1</td>
<td>49.8 ± 9.7</td>
<td>0.581</td>
</tr>
<tr>
<td>Large LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>264.7 ± 47.5</td>
<td>273.0 ± 47.0</td>
<td>0.722</td>
</tr>
<tr>
<td>Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1480.0 ± 111.2</td>
<td>1506.3 ± 107.5</td>
<td>0.534</td>
</tr>
<tr>
<td>Medium Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>312.4 ± 25.0</td>
<td>307.7 ± 24.2</td>
<td>0.755</td>
</tr>
<tr>
<td>Very Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1167.6 ± 86.9</td>
<td>1198.4 ± 85.3</td>
<td>0.355</td>
</tr>
<tr>
<td>LDL Particle Size</td>
<td>nm</td>
<td>20.1 ± 0.1</td>
<td>20.1 ± 0.1</td>
<td>0.385</td>
</tr>
<tr>
<td>oLDL</td>
<td>µg·mL⁻¹</td>
<td>1476.2 ± 89.2</td>
<td>1243.2 ± 479.1</td>
<td>0.490</td>
</tr>
</tbody>
</table>

All values are means ± SEM. * = Significant difference between conditions (p < 0.05).
Baseline, Before exercise (Control); 24 hours post-exercise; VLDL, Very Low-Density Lipoprotein; LDL, Low-Density Lipoprotein, oLDL, oxidized Low-Density Lipoprotein.

The acute bout of exercise did not alter large VLDL particle numbers ($F_{1,11} = 0.54, p = 0.321$) and medium VLDL particles numbers ($F_{1,11} = 1.30, p = 0.222$).

However, the small VLDL particles were significantly increased ($F_{1,11} = 0.51, p = 0.016*$).

An acute bout of exercise did not cause a change in total LDL particle numbers ($F_{1,11} = 1.34, p = 0.272$), intermediate LDL particle numbers ($F_{1,11} = 0.32, p = 0.581$), large LDL particle numbers ($F_{1,11} = 0.13, p = 0.722$), small LDL particle numbers ($F_{1,11} = 0.41, p = 0.534$), medium small LDL particle numbers ($F_{1,11} = 0.10, p = 0.755$), and very small LDL particle numbers ($F_{1,11} = 0.93, p = 0.355$). An acute bout of exercise did not
alter MPO ($F_{1,11} = 1.56$, $p = 0.238$). An acute bout of exercise did not alter the particle sizes of VLDL ($F_{1,11} = 1.74$, $p = 0.102$) and LDL ($F_{1,11} = 0.82$, $p = 0.385$). Additionally, acute exercise did not change the concentration of oLDL ($F_{1,11} = 0.51$, $p = 0.490$).

**Correlations Before and After Exercise Intervention**

Pearson product-moment correlations were calculated to gain additional insight into the relationships between PON1 activity and concentration and measures of lipoproteins and oxidative stress.

There were no significant correlations between PON1 activity and any of the other variables of interest. PON1 concentration was only correlated with Apo A1 ($r = 0.595$, $p = 0.041^*$).

Changes in variables of interest occurring with exercise (24 hours post-exercise – baseline) were calculated and the relationship between baseline PON1 activity and concentration and change variables were determined. Baseline PON1 activity correlated significantly with PON1 activity change ($r = -0.584$, $p = 0.046^*$), Apo A1 change ($r = -0.671$, $p = 0.017^*$), and MPO change ($r = -0.611$, $p = 0.035^*$). PON1 concentration correlated with LDL particle number change ($r = -0.607$, $p = 0.036^*$) and small LDL particle number change ($r = -0.585$, $p = 0.046^*$).

Change variables for exercise were computed for relationships with PON1 activity change and PON1 concentration change. The exercised-induced change in PON1 activity was significantly correlated with changes in HDLc ($r = 0.740$, $p = 0.006^*$). Changes in PON1 concentration was not correlated with changes in any of the variables of interest.
Six Weeks of Niacin Therapy

The influence of extended-release niacin on the variables of interest was determined by comparing baseline measures with 6-weeks post-niacin (Niacin_Pre) prior to completing a second bout of exercise. The variables of interest responses to 6-weeks of extended-release niacin therapy as compared to baseline are presented in Table 10.

Table 10 – Response to niacin therapy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Baseline</th>
<th>Niacin_Pre</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1a</td>
<td>kU·L⁻¹</td>
<td>125.9 ± 4.9</td>
<td>115.9 ± 6.2</td>
<td>0.143</td>
</tr>
<tr>
<td>PON1c</td>
<td>µg·mL⁻¹</td>
<td>112.4 ± 8.2</td>
<td>105.9 ± 8.5</td>
<td>0.536</td>
</tr>
<tr>
<td>Apo A1</td>
<td>µg·mL⁻¹</td>
<td>5031.5 ± 1646.1</td>
<td>4339.3 ± 1061.1</td>
<td>0.198</td>
</tr>
<tr>
<td>Total HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>31.5 ± 1.3</td>
<td>32.0 ± 1.1</td>
<td>0.775</td>
</tr>
<tr>
<td>Large HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>3.5 ± 0.5</td>
<td>3.8 ± 0.8</td>
<td>0.875</td>
</tr>
<tr>
<td>Medium HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>5.1 ± 1.5</td>
<td>2.9 ± 0.9</td>
<td>0.146</td>
</tr>
<tr>
<td>Small HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>22.9 ± 1.4</td>
<td>25.3 ± 1.1</td>
<td>0.313</td>
</tr>
<tr>
<td>Total HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>31.5 ± 1.3</td>
<td>32.0 ± 1.1</td>
<td>0.775</td>
</tr>
<tr>
<td>HDL Particle Size</td>
<td>nm</td>
<td>8.6 ± 0.1</td>
<td>8.6 ± 0.1</td>
<td>0.862</td>
</tr>
<tr>
<td>HDLc</td>
<td>mg·dL⁻¹</td>
<td>41 ± 2</td>
<td>41 ± 2</td>
<td>0.690</td>
</tr>
<tr>
<td>TG</td>
<td>mg·dL⁻¹</td>
<td>293 ± 28</td>
<td>196 ± 19</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

All values are means ± SEM. * = Significant difference between conditions (p < 0.05). Baseline, Before exercise (Control); Niacin_Pre, 6-weeks of niacin before exercise; PON1a, Paraoxonase 1 Activity; PON1c, Paraoxonase 1 Concentration; Apo A1, Apolipoprotein A1; HDL, High-Density Lipoprotein; HDLc, High-Density Lipoprotein cholesterol; TG, Triglyceride concentration.

Six weeks of niacin therapy did not alter PON1 activity (F₁,₁₁ = 2.49, p = 0.143).

Additionally, six weeks of niacin therapy did not alter PON1 concentration (F₁,₁₁ = 0.41, p = 0.536). Six weeks of niacin therapy did not alter Apo A1 (F₁,₁₁ = 1.90, p = 0.198).

Extended-release niacin had no effect on the total HDL particle numbers (F₁,₁₁ = 0.09, p = 0.775), large HDL particle numbers (F₁,₁₁ = 0.03, p = 0.875), medium HDL particle numbers (F₁,₁₁ = 2.49, p = 0.146), and small HDL particle numbers (F₁,₁₁ = 1.13,
Six weeks of niacin therapy did not alter HDL particle size ($F_{1,11} = 0.18, p = 0.862$). Six weeks of niacin did not alter HDLc ($F_{1,11} = 0.169, p = 0.690$). Six weeks of niacin significantly reduced TG ($F_{1,11} = 26.55, p < 0.001^*$).

The VLDL particle numbers, VLDL particle size, LDL particle numbers, LDL particle size, and oLDL responses to extended-release niacin are listed in Table 11.

### Table 11 – VLDL & LDL response to niacin therapy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Baseline</th>
<th>Niacin_Pre</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large VLDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>5.7 ± 1.0</td>
<td>5.4 ± 0.8</td>
<td>0.745</td>
</tr>
<tr>
<td>Medium VLDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>31.5 ± 5.3</td>
<td>25.4 ± 3.7</td>
<td>0.185</td>
</tr>
<tr>
<td>Small VLDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>48.6 ± 3.8</td>
<td>44.6 ± 5.8</td>
<td>0.317</td>
</tr>
<tr>
<td>VLDL Particle Size</td>
<td>nm</td>
<td>58.4 ± 2.4</td>
<td>59.9 ± 3.0</td>
<td>0.368</td>
</tr>
<tr>
<td>Total LDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>1788.3 ± 105.1</td>
<td>1626.1 ± 131.6</td>
<td>0.129</td>
</tr>
<tr>
<td>Intermediate LDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>43.6 ± 13.1</td>
<td>32.1 ± 13.7</td>
<td>0.492</td>
</tr>
<tr>
<td>Large LDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>264.7 ± 47.5</td>
<td>349.4 ± 65.7</td>
<td>0.360</td>
</tr>
<tr>
<td>Small LDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>1480.0 ± 111.2</td>
<td>1244.6 ± 128.8</td>
<td>0.057</td>
</tr>
<tr>
<td>Medium Small LDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>312.4 ± 25.0</td>
<td>244.6 ± 26.3</td>
<td>0.018*</td>
</tr>
<tr>
<td>Very Small LDL Particles</td>
<td>nmol·L$^{-1}$</td>
<td>1167.6 ± 86.9</td>
<td>1000.0 ± 103.5</td>
<td>0.079</td>
</tr>
<tr>
<td>LDL Particle Size</td>
<td>nm</td>
<td>20.1 ± 0.1</td>
<td>20.3 ± 0.2</td>
<td>0.339</td>
</tr>
<tr>
<td>oLDL</td>
<td>µg·mL$^{-1}$</td>
<td>1476.2 ± 89.2</td>
<td>1056.8 ± 353.9</td>
<td>0.284</td>
</tr>
</tbody>
</table>

All values are means ± SEM. * = Significant difference between conditions (p < 0.05). Baseline, Before exercise (Control); Niacin_Pre, 6-weeks of niacin before exercise; VLDL, Very Low-Density Lipoprotein; LDL, Low-Density Lipoprotein; oLDL, Oxidized Low-Density Lipoprotein Concentration.

Extended-release niacin did not alter large VLDL particle numbers ($F_{1,11} = 0.34, p = 0.745$), medium VLDL particle numbers ($F_{1,11} = 1.42, p = 0.185$), and small VLDL particle numbers ($F_{1,11} = 1.05, p = 0.317$). Six weeks of extended-release niacin therapy did not alter VLDL particle size ($F_{1,11} = 0.94, p = 0.368$).

Total LDL particle numbers ($F_{1,11} = 2.74, p = 0.129$), intermediate LDL particle numbers ($F_{1,11} = 0.51, p = 0.492$), large LDL particle numbers ($F_{1,11} = 0.92, p = 0.360$),
small LDL particle numbers ($F_{1,11} = 4.65, p = 0.057$), and very small LDL particle numbers ($F_{1,11} = 3.81, p = 0.079$) remained unchanged following niacin therapy. However, medium small LDL particle numbers were significantly reduced following niacin therapy ($F_{1,11} = 8.08, p = 0.018^*$$). Six weeks of extended-release niacin therapy did not alter LDL particle size ($F_{1,11} = 1.01, p = 0.339$).

Niacin did not alter MPO as compared to baseline ($F_{1,11} = 1.37, p = 0.267$). Nor did it change the concentration of oLDL from baseline ($F_{1,11} = 1.27, p = 0.284$).

**Correlations Before and After 6-weeks of Niacin Therapy**

Pearson Product-Moment Correlations were calculated to determine relationships between PON1 activity and concentration and measures of lipoproteins and oxidative stress following six weeks of niacin therapy. The baseline correlations have been previously reported.

Changes in variables of interest occurring with niacin therapy (6-weeks of niacin therapy – baseline) were calculated and the relationships between baseline PON1 activity and concentration and change variables were determined. Baseline PON1 activity was not correlated with any of the change variables. Baseline PON1 concentration was correlated with changes in PON1 concentration ($r = -0.796, p = 0.002^*$$) and changes in small LDL particle numbers ($r = -0.616, p = 0.043^*$$). PON1 concentration was not correlated with any other change variables.

Change variables following niacin therapy were computed for relationships with changes in PON1 activity and concentration. There were no significant correlations in the changes in PON1 activity with any other change variable. Niacin-induced changes PON1 concentration was significantly correlated with changes in LDL particle number ($r = 0.775, p = 0.005^*$$) and changes in small LDL particle numbers ($r = 0.878, p < 0.001^*$$).
Combined Effects of Exercise and Niacin Therapy

The influence of the combined effects of acute exercise and extended-release niacin therapy on the variables of interest was determined by comparing 6-weeks of niacin therapy before exercise (Niacin) with measures following an acute bout of exercise after 6-weeks of niacin therapy (Niacin + Exercise). PON1 activity and PON1 concentration responses to the combined effects of acute exercise and 6-weeks of extended-release niacin therapy are presented in Figure 3 and 4.

Figure 3 – PON1 activity response to exercise and niacin therapy.

![Figure 3](image)

PON1 activity is presented as means ± SD; kU/L, kilo international units per liter of sample; * = Significant difference between conditions (p < 0.05); Baseline (control), before exercise; Exercise, 24 hours post-exercise; Niacin, 6 weeks of niacin therapy; Niacin + Exercise, 24 hours post-exercise plus niacin.

The PON1 activity was significantly increased following the combined effects of acute exercise and extended-release niacin ($F_{1,11} = 5.67, p = 0.037$) (Figure 3).
Figure 4 – PON1 concentration response to exercise and niacin therapy.

PON1 concentration is presented as means ± SD; µg/mL, micrograms per milliliter of sample; * = Significant difference between conditions (p < 0.05); Baseline (control), before exercise; Exercise, 24 hours post-exercise; Niacin, 6 weeks of niacin therapy; Niacin + Exercise, 24 hours post-exercise plus niacin.

PON1 concentration was significantly increased ($F_{1,11} = 8.25$, $p = 0.015$) (Figure 4).

Table 12 represents the responses of Apo A1, HDL particle number, HDL particle size, HDLc, and TG to the combined effects of acute exercise and extended-release niacin.
Table 12 – Response to exercise and niacin therapy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Niacin_Pre</th>
<th>Niacin_AEX</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A1</td>
<td>µg·mL⁻¹</td>
<td>4339.3 ± 1061.1</td>
<td>3871.2 ± 2839.0</td>
<td>0.059</td>
</tr>
<tr>
<td>Total HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>32.0 ± 1.1</td>
<td>33.2 ± 1.3</td>
<td>0.102</td>
</tr>
<tr>
<td>Large HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>3.8 ± 0.8</td>
<td>3.8 ± 0.6</td>
<td>0.807</td>
</tr>
<tr>
<td>Medium HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>2.9 ± 0.9</td>
<td>3.9 ± 0.7</td>
<td>0.250</td>
</tr>
<tr>
<td>Small HDL Particles</td>
<td>µmol·L⁻¹</td>
<td>25.3 ± 1.1</td>
<td>25.5 ± 1.4</td>
<td>0.846</td>
</tr>
<tr>
<td>HDL Particle Size</td>
<td>nm</td>
<td>8.6 ± 0.1</td>
<td>8.6 ± 0.1</td>
<td>0.831</td>
</tr>
<tr>
<td>HDLc</td>
<td>mg·dL⁻¹</td>
<td>41 ± 2</td>
<td>43 ± 2</td>
<td>0.124</td>
</tr>
<tr>
<td>TG</td>
<td>mg·dL⁻¹</td>
<td>196 ± 19</td>
<td>159 ± 12</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

All values are means ± SEM. * = Significant difference between conditions (p < 0.05).
Niacin_Pre, Before exercise; Niacin_AEX, 24 hours post exercise with niacin. Apo A1, Apolipoprotein A1; MPO, Myeloperoxidase Concentration; oLDL, Oxidized Low-Density Lipoprotein Concentration; HDL, High-Density Lipoprotein; HDLc, High-Density Lipoprotein cholesterol; TG, Triglyceride concentration.

Acute exercise combined with 6 weeks of niacin therapy did not alter Apo A1 (F₁,₁₁ = 4.85, p = 0.059). HDL particle number responses to the combined effects of acute exercise and 6 weeks of niacin therapy are listed below in Table 12. These two interventions did not alter total HDL particle numbers (F₁,₁₁ = 3.25, p = 0.102). The large HDL particle numbers were not altered (F₁,₁₁ = 0.06, p = 0.807). The medium HDL particle numbers were not altered (F₁,₁₁ = 1.49, p = 0.250). The same treatments did not alter the small HDL particle numbers (F₁,₁₁ = 0.04, p = 0.846). HDL particle size was not altered (F₁,₁₁ = 0.05, p = 0.831). The combined effects of acute exercise and niacin therapy did not significantly alter HDLc (F₁,₁₁ = 2.825, p = 0.124). TG were significantly reduced following the combined effects of exercise and niacin therapy (F₁,₁₁ = 11.70, p = 0.006*).
The VLDL particle numbers, VLDL particle size, LDL particle numbers, LDL particle size, and oLDL responses to the combined effects of acute exercise and niacin therapy are presented below in Table 13.

**Table 13 – VLDL & LDL response to exercise and niacin therapy.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Niacin_Pre</th>
<th>Niacin_AEX</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>5.4 ± 0.8</td>
<td>4.2 ± 1.0</td>
<td>0.043*</td>
</tr>
<tr>
<td>Medium VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>25.4 ± 3.7</td>
<td>25.9 ± 4.0</td>
<td>0.749</td>
</tr>
<tr>
<td>Small VLDL Particles</td>
<td>nmol·L⁻¹</td>
<td>44.6 ± 5.8</td>
<td>49.4 ± 4.8</td>
<td>0.143</td>
</tr>
<tr>
<td>VLDL Particle Size</td>
<td>nm</td>
<td>59.9 ± 3.0</td>
<td>55.5 ± 2.9</td>
<td>0.032*</td>
</tr>
<tr>
<td>Total LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1626.1 ± 131.6</td>
<td>1678.0 ± 121.1</td>
<td>0.325</td>
</tr>
<tr>
<td>Intermediate LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>32.1 ± 13.7</td>
<td>38.9 ± 12.8</td>
<td>0.455</td>
</tr>
<tr>
<td>Large LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>349.4 ± 65.7</td>
<td>352.1 ± 51.8</td>
<td>0.935</td>
</tr>
<tr>
<td>Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1244.6 ± 128.8</td>
<td>1287.4 ± 110.1</td>
<td>0.474</td>
</tr>
<tr>
<td>Medium Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>244.6 ± 26.3</td>
<td>266.4 ± 24.7</td>
<td>0.204</td>
</tr>
<tr>
<td>Very Small LDL Particles</td>
<td>nmol·L⁻¹</td>
<td>1000.0 ± 103.5</td>
<td>1021.1 ± 86.3</td>
<td>0.649</td>
</tr>
<tr>
<td>LDL Particle Size</td>
<td>nm</td>
<td>20.3 ± 0.2</td>
<td>20.4 ± 0.1</td>
<td>0.700</td>
</tr>
<tr>
<td>oLDL</td>
<td>µg·mL⁻¹</td>
<td>1056.8 ± 353.9</td>
<td>1459.0 ± 451.7</td>
<td>0.290</td>
</tr>
</tbody>
</table>

All values are means ± SEM. * = Significant difference between conditions (p < 0.05). Niacin_Pre, Before exercise; Niacin_AEX, 24 hours post exercise with niacin. VLDL, Very Low-Density Lipoprotein; LDL, Low-Density Lipoprotein; oLDL, oxidized Low-Density Lipoprotein.

The combined effects of acute exercise and extended-release niacin significantly reduced large VLDL particle numbers (F₁,₁₁ = 2.312, p = 0.043*). However, these two interventions did not alter medium VLDL particle numbers (F₁,₁₁ = 0.33, p = 0.749), and small VLDL particle numbers (F₁,₁₁ = 1.59, p = 0.143).

The interventions of exercise and niacin therapy did not change the particle numbers of total LDL particles numbers (F₁,₁₁ = 1.07, p = 0.325), intermediate LDL particle numbers (F₁,₁₁ = 0.61, p = 0.455), large LDL particle numbers (F₁,₁₁ = 0.01, p = 0.935), small LDL particle numbers (F₁,₁₁ = 0.55, p = 0.474), medium small LDL particle sizes.
numbers ($F_{1,11} = 1.85$, $p = 0.204$), and very small LDL particle numbers ($F_{1,11} = 0.22$, $p = 0.649$).

Lipoprotein particle size responses to the combined effects of acute exercise and niacin therapy are listed below in table 13. The combined interventions significantly reduced VLDL particle size ($F_{1,11} = 2.48$, $p = 0.032^*$). However, LDL particle size was not altered ($F_{1,11} = 0.16$, $p = 0.700$).

Acute exercise along with 6 weeks of niacin therapy did not alter MPO ($F_{1,11} = 0.28$, $p = 0.605$). The same treatments did not alter oLDL ($F_{1,11} = 1.24$, $p = 0.290$).

**Correlations Following the Combined Effects of Exercise and Niacin Therapy**

Pearson product-moment correlations were calculated to gain additional insight into the relationships between PON1 activity and concentration and measures of lipoproteins and oxidative stress.

There were no significant correlations between PON1 activity and any of the other variables of interest following the combined effects of acute exercise and niacin therapy. Baseline PON1 concentrations were significantly correlated with baseline LDL particle numbers ($r = 0.733$, $p = 0.010^*$) and small LDL particle numbers ($r = 0.677$, $p = 0.022^*$) following the combined interventions of exercise and niacin therapy.

Changes in variables of interest occurring with exercise and niacin therapy (6-Weeks Niacin Therapy plus exercise – baseline with niacin only) were calculated and the relationships between baseline PON1 activity and concentration and change variables were determined. Baseline PON1 activity was significantly correlated with changes in HDL particle size ($r = 0.748$, $p = 0.008^*$). Baseline PON1 activity was not correlated with any other change variables. Baseline PON1 concentrations were significantly correlated with changes in PON1 activity ($r = -0.688$, $p = 0.013^*$). Baseline PON1 concentrations were not correlated with any other change variables.
Exercise and niacin induced changes in PON1 activity was correlated with changes in HDL particle size ($r = -0.627$, $p = 0.039^*$). Changes in PON1 concentration was not correlated with any change variable following the combined effects of exercise and niacin therapy.
CHAPTER V.
DISCUSSION

The overall purpose of this study was to examine the independent and combined effects of acute exercise and 6-weeks of extended release niacin therapy on PON1 concentration and activity in men with MetS. The primary and novel findings of this study are that exercise and niacin together increased PON1 concentration and activity, but these PON1 characteristics were not altered by either intervention alone. Our results indicate for the first time that these therapeutic interventions have additive or complementary effects on PON1 concentration and activity in those with metabolic dyslipidemia. Furthermore, PON1 concentration and activity can be elevated in the absence of changes in markers of oxidative stress and changes in lipoprotein lipids that are often reported with either exercise or niacin.

The participants were middle-aged and categorized as obese according to BMI [295]. The average waist circumference would place the cohort at increased risk for metabolic and cardiovascular disease [296]. Body fat percentage would place the group into the lowest 10th percentile among age-matched men [295]. The relative maximal oxygen uptake is lower than all but 10% of age-matched men [295]. HDLc for this cohort averaged 41 ± 14 mg·dL⁻¹, which is on the low end of the reference values for men [1]. All participants met criteria for MetS as defined by NCEP ATP III [1]. All participants were hypertriglyceridemic and met clinical standards for extended-release niacin prescription [98].
Effect of Exercise on PON1 Concentration and Activity

We hypothesized that one exercise session would increase HDLc and increase PON1 concentration and activity. Furthermore, markers of lipid peroxidation, oLDL and MPO, were hypothesized to increase following an acute bout of exercise. The results of this investigation do not support our original hypotheses regarding the independent effects of exercise. PON1 concentration and activity, Apo A1, MPO, oLDL, and HDLc were not altered following a single session of aerobic exercise.

Dynamic continuous exercise has been previously shown to increase PON1 activity [94, 95] with some exceptions [236, 237]. The exercise effects on PON1 activity appear to be transient as we and others [94, 95, 237] do not observe significant elevations 24 hours after completing exercise. Tomas et al. [94] found that PON1 activity and oLDL was significantly increased following 30 minutes of cycle ergometry in previously sedentary individuals who completed 16-weeks of aerobic exercise training. The post-training results of the exercise session were different from what was observed prior to training. It may be argued, based on the findings of Tomas et al. [94] that long-term exercise training may be necessary for PON1 changes to take place. In other words, systemic, cellular, and molecular changes induced by regular practiced exercise may be necessary for increasing PON1. Limited support for this position may be found from cross-sectional results where adolescents that participated in sports had a higher PON1 activity than their sedentary counterparts [92, 93]. Evidence from cellular adaptations to regular exercise appear to be consistent with this position. Training adaptations increase skeletal muscle PKC [297] and PKC along with Sp1 has been found to increase the expression of PON1 [45].

Increases in HDLc and decreases in TG are common following a single bout of exercise intensities between 70% and 80% while expending 300 to 500 kcals. These
changes are maintained up to 48 hours post-exercise [81, 82, 123]. However, HDLc was not altered in this current investigation.

Although, there are different methods to quantify the HDL particle sizes. HDL$_3$ is generally obtained through precipitation of Apo B-containing lipoproteins from serum or plasma. HDL$_3$ would be considered the smaller of the HDL subfractions. The HDL$_3$ subclass has a PON1 activity 25 times higher than the larger HDL$_2$ subclass [211]. Although HDL particle sizes were determined by NMR, it appears that exercise in our investigation resulted in smaller HDL particles without observed changes in Apo A1 or LDL characteristics and in the absence of changes in PON1 concentration or activity. In addition, PON1 concentration and activity were not correlated with exercise-induced changes in HDL particle size. Our findings may be interpreted to mean that the reduction in HDL particle size does not necessarily result in characteristic changes in PON1. However, reduction in HDL particle size may contribute to subsequent increases in PON1 concentration and activity.

**Effect of Niacin on PON1 Concentration and Activity**

Our second hypothesis was that six weeks of niacin therapy alone would increase the concentration and activity of PON1 and reduce markers of lipid peroxidation. The means by which niacin may induce changes in PON1 has not been investigated. Some possibilities include reductions in the production of oLDL, the formation of MPO and/or malondialdehyde [103, 114]. Statins are known to increase PON1 expression through activation of PPAR-γ [119-121]. Niacin is capable of activating the PPAR-γ pathway [122]; however, PON1 expression has not been studied with niacin therapy. Our results indicate that niacin doses up to 1500 mg·day$^{-1}$ for 6 weeks does not appear to affect PON1, HDL, Apo A1, or blood markers of lipid
peroxidation. To our knowledge, ours is the first study designed to examine PON1 changes with niacin therapy.

Niacin has been used as an effective therapy for increasing HDLc and lowering TG concentrations [98, 116, 278, 298]. Our results are consistent with those published previously regarding the TG-lowering effects of niacin [98, 116, 278, 298]. We found that HDL particles and HDLc were not altered in our investigation. HDL particle numbers remained static throughout our niacin intervention. There are several groups that have examined particle size distribution following niacin therapy [283, 299-301]. In contrast, Kuvin et al. [283], examining individuals with stable coronary artery disease, reported 12 weeks of niacin therapy at a dosage of 1000 mg·day⁻¹ significantly increased large HDL particle numbers and significantly decreased small HDL particle numbers. Similar to Kuvin, Jafri et al. [300] titrated niacin to a dosage of 1000 mg·day⁻¹ over 12 weeks and reported a similar significant increase in large HDL particle numbers and a decrease in small HDL particle numbers in individuals with stable coronary artery disease. In addition, Shearer et al. [301] reported that 16-weeks of niacin therapy at a dosage of 2000 mg·day⁻¹ elicited a significant increase in the large HDL particles in individuals with MetS.

Niacin increases the production of Apo A1 as compared to placebo [302]. Apo A1 improves the ability of HDL to promote cholesterol efflux from peripheral tissues [303] and Apo A1 plays a vital role at increasing the activity of PON1 [28]. Lamon-Fava et al. [302] found that extended-release niacin titrated over 12 weeks at a dosage of 2000 mg·day⁻¹ would cause a significant increase in Apo A1. However, in our current investigation, Apo A1 was not altered following 6 weeks of niacin therapy at a dosage of 1500 mg·day⁻¹.

MPO is a byproduct of lipoprotein lipid oxidation, and MPO may reduce the protective effects of HDL to maintain vascular function of the endothelium [114]. On the
other hand, niacin is thought to improve vascular health by reducing oxidative stress [114, 304]. Sorrentino and colleagues [114] found a significant reduction in MPO following 12 weeks of niacin therapy titrated to 1500 mg·day\(^{-1}\) in individuals with T2DM and meeting criteria for MetS. MPO was not altered following 6 weeks of niacin therapy in the present study.

Although, extended-release niacin therapy reduced total and small LDL particle numbers by 10% to 18% and increased large LDL particles by 32% in our study, these changes were not significant. Jaffri et al. [300] found that extended-release niacin significantly reduced total and small LDL particle numbers by 10% to 15% in 27 individuals with stable coronary artery disease. Kuvin et al. [283] found that extended-release niacin significantly reduced small LDL particles by 12%. Kuvin and colleagues [283] reported a significant 82% increase in the large LDL particles. We did not observe changes in PON1 concentration or activity, but changes in lipoprotein metabolism, decreased TG and decreased LDL particle distribution, are consistent with previously reported changes with niacin. The reduction in small LDL particles and the increase in the large LDL particles are consistent with a shift toward a less atherogenic LDL profile following six weeks of niacin therapy. The changes that we observed may be - and are likely - contributory to changes in PON1 concentration and activity observed with combination of exercise and niacin therapy.

**Effect of Exercise and Niacin Therapy on PON1 Concentration and Activity**

The combined effects of exercise and niacin were thought to induce changes in PON1 and markers of oxidative stress above what was expected by either intervention alone, because each of these interventions potentially involves different but potentially complimentary physiological processes. With respect to PON1 characteristics, niacin influences on hepatic, adipose, or skeletal muscle tissue - some of which have been
discussed previously - may be similar to that observed after regularly-practiced exercise. It is possible that the dual interventions of exercise and niacin therapy complement one another to increase PON1 concentration and activity because we observed significant increases in both after combining these strategies.

Interestingly, transient changes were observed 24 hours after exercise. This has not been observed in previous studies. We interpret this to mean that niacin exerts an influence on exercise response that persists beyond what has been shown for exercise alone. Increases in PON1 concentration and activity may be attributed to the activation of both PKC and PPAR-\(\gamma\) at the cellular or molecular level or to changes in lipoproteins in circulation. However, these postulated mechanisms have yet to be confirmed by experimental data.

The combination of exercise and niacin therapy on lipoprotein particle size distribution has not been studied. Large VLDL particle size are considered to be a major contributor to abnormal lipoprotein metabolism [305]. The combination of exercise and extended-release niacin therapy significantly decreased VLDL particle numbers by 8%. The greatest change was found with VLDL particle size, which decreased by 28% following the dual intervention. Independently, exercise and niacin have been shown to decrease the availability of hepatic TG to incorporate into VLDL and results in a reduction of VLDL particle size [82, 116, 306]. Furthermore, there is less availability of TG in the peripheral circulation to exchange TG for cholesterol in HDL and LDL leading to increased particle size for both HDL and LDL [275, 307]. Another key action of niacin results in decreased activity of CETP, which may be a factor to increase the size of the HDL particle [308, 309]. Results from these studies may help us understand how exercise and niacin work together. Our results suggest that exercise and niacin appear to have a complimentary effect on VLDL particle distribution.
Our findings confirm our hypothesis that the combined effects of exercise and niacin increased PON1 concentration and activity above either intervention alone. PON1 concentrations increased 12.3% following the combination of exercise and extended-release niacin \((p = 0.015)\). PON1 activity increased 6% with the combination of exercise and extended-release niacin therapy \((p = 0.037)\). PON1 concentration and activity increased without observed changes in Apo A1, oLDL, and MPO.

**Conclusion**

PON1 can protect lipoproteins from oxidative modification by protecting against oxidative stress and inflammation. The proposed mechanism for PON1 against oxidative stress appears to be an interaction between ROS and the free sulfhydryl group at cysteine-284 [178, 233]. As such, PON1 concentration and/or activity may be important and often overlooked characteristics of HDL to decrease an individual’s risk for CVD either through lifestyle modification or through pharmacologic intervention. The independent actions of exercise did not alter PON1 concentration and activity. Similarly, 6-weeks of extended-release niacin therapy did not alter PON1 concentration and activity. However, when exercise was completed after 6 weeks of extended-release niacin therapy, PON1 concentration and activity significantly increased without observed changes in oLDL and MPO. MPO and oLDL were unaltered following the independent or combined interventions of an acute bout of exercise or six weeks of extended-release niacin therapy. Niacin doses of 1000 to 2000 mg·day\(^{-1}\) appear to have a greater effect on HDL and LDL particles over 12 weeks than those studies of shorter duration, suggesting a dose or duration issue similar to dose/duration thresholds observed with exercise.
Limitations

Our investigation was limited to sedentary middle-aged men with MetS. Responses to single sessions of exercise at an intensity of 60 to 70% \( \text{VO}_2\text{max} \) were measured and participants remained sedentary throughout the study. We only measured responses before niacin therapy and following six weeks of niacin therapy (pre and post-exercise).

Future Directions

The physiological, cellular, and molecular means by which exercise influences PON1 are poorly understood. There is a need to understand modulators of PON1. Future research may characterize and compare dietary influences, exercise, niacin, weight reduction, and other therapeutic interventions on PON1. Progressive exercise training studies employed to investigate PON1 activity and concentration or extending the duration or dosage of niacin therapy might result in greater PON1 responses. However, with both exercise and niacin, compliance becomes an issue when extending duration or increasing dosages and/or intensities, especially with unfit individuals. We demonstrated that exercise plus niacin are capable of increasing PON1. However, it is important to note that increases in PON1 activity and concentration have not been shown to represent a physiologically-meaningful potentiation in antioxidant status. In addition, it is not known if increases in PON1 activity and concentration represent a decreased CVD risk.
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