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 $kg \cdot m^2$; % fat = 35 ± 5; $\dot{V}O_{2max}$ = 27.5 ± 6.6 $mL \cdot min^{-1} \cdot kg^{-1}$; waist circumference = 108.8 ± 8.2 cm; HDL-C = 39 ± 8 and triglycerides = 287 ± 96 $mg \cdot dL^{-1}$; HOMA score = 4.4 ± 2.0) expended 500 kcals by treadmill walking at 65% of $\dot{V}O_{2max}$ before and after a 6-week regimen of prescription niacin. Niacin doses were titrated by 500 $mg \cdot wk^{-1}$ from 500 to 1500 $mg \cdot dy^{-1}$ and maintained at 1500 $mg \cdot dy^{-1}$ 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 $nmol \cdot L^{-1}$) following six weeks of niacin therapy (p = 0.057). PON1 activity increased 6.1% (from 115.9 ± 24.0 to 122.9 ± 19.8 $kU \cdot L^{-1}$; p = 0.037*) and PON1 concentrations increased 11.3% (from 109.5 ± 24.3 to 121.9 ± 19.8 $ug \cdot mL^{-1}$; 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 $mg \cdot dL^{-1}$; high density lipoprotein concentration (HDLc) ≤ 40 $mg \cdot dL^{-1}$ for men and ≤ 50 $mg \cdot dL^{-1}$ for women; systolic blood pressure $\geq$ 130 and/or diastolic blood pressure $\geq$ 85 mmHg, and; fasting plasma glucose levels ≥ 100 $mg \cdot dL^{-1}$ [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 $mg \cdot dL^{-1}$ 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 ($\dot{V}O_{2\ max}$) 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 $mg \cdot day^{-1}$) 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 $mg \cdot day^{-1}$) over 25 weeks was effective at increasing HDLc by 10% to 30% in a dose dependent fashion up to 2500 to 3000 $mg \cdot day^{-1}$. The favorable increase in HDLc by extended-release niacin appears to be a result of its ability to increase HDL_2 subfraction [110]. The HDL_2 subfraction has antioxidant properties, inhibits thrombotic factors, and is considered to be more cardioprotective of the HDL subfractions [111, 112]. The increase in HDL_2 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 $mg \cdot dL^{-1}$. 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% $\dot{V}O_{2max}$ 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% $\dot{V}O_{2max}$ 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 $mg \cdot day^{-1}$, 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 PON 1 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% $\dot{V}O_{2max}$
- Participants were prescribed extended release niacin that was titrated over a 3-week period from 500 $mg \cdot day^{-1}$ (week 1) to 1500 $mg \cdot day^{-1}$ (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/m^2] and an even larger proportion (36%) were obese (BMI ≥ 30.0 kg/m^2) [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 ≥ 150 $mg \cdot dL^{-1}$ and cholesterol ≥ 200 $mg \cdot dL^{-1}$ along with low levels of plasma HDLc ≤ 40 $mg \cdot dL^{-1}$ for men and ≤ 50 $mg \cdot dL^{-1}$ 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 it antiatherogenic properties [20, 21]. MPO is released from phagocytes that respond to areas that have chronic inflammation [22] and MPO uses hydrogen peroxide (H_2O_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 sulfhydril 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-γ), and sterol-responsive element binding protein-2 (SREBP-2) (Figure 1) [45-47].

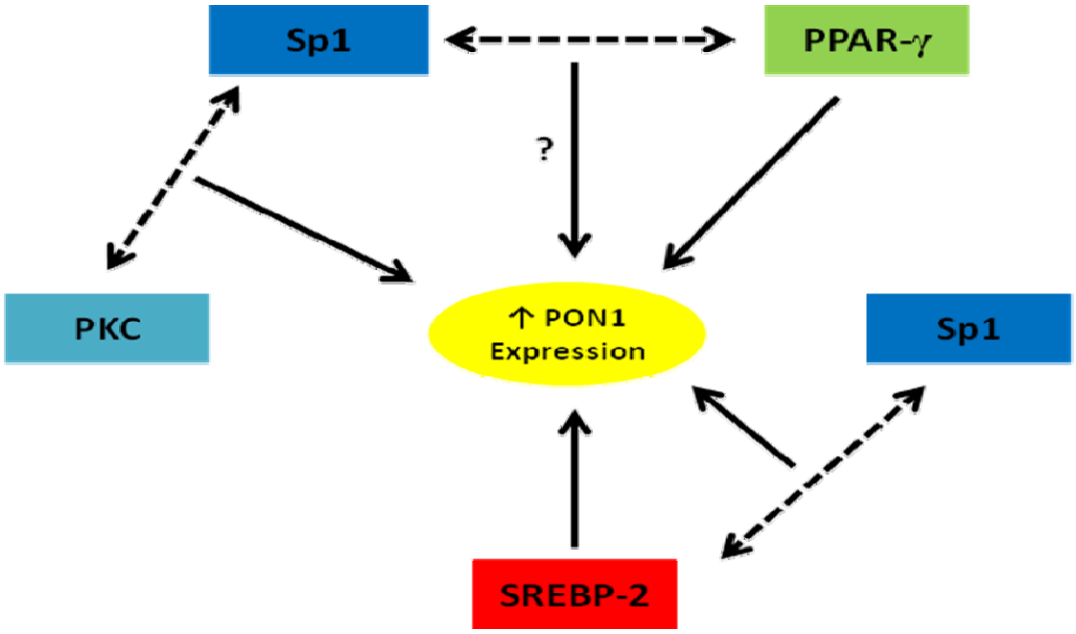
The PON1 gene is regulated by Sp1. Sp1 acts through the promoter GC boxes to up- or downregulate genes [185]. Sp1 activities are increased during maturation of monocytes into macrophages and during adipocytes differentiation [186]. Chronically high glucose concentrations are a key factor that activates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

PPAR-γ is expressed highest in adipose tissues (white and brown adipose tissue) and activated macrophages, including the foam cells of atherosclerotic lesions [188-191]. PPAR-γ 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-γ in macrophages and monocytes [195]. Activation of PPAR-γ results in improved insulin sensitivity and enhanced glucose disposal in adipose tissue and skeletal muscle [196]. The mechanism in which activation of PPAR-γ 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.

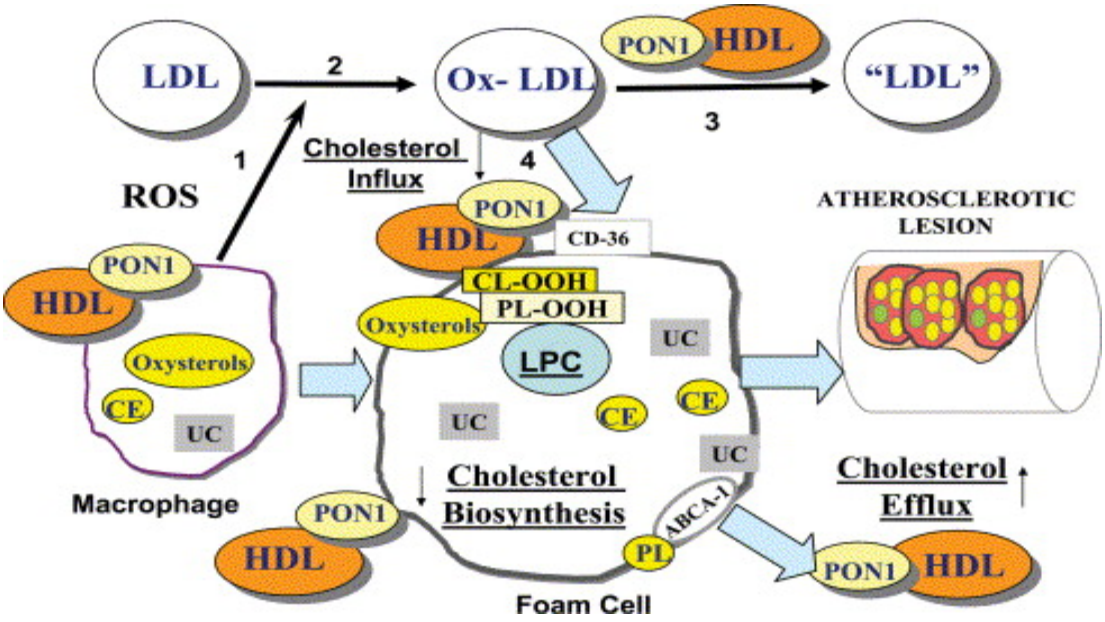
PON1 Activity and Concentration

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 anti-atherogenic.

PON1 inhibits the macrophage from releasing superoxide anions, which results in a decreased oxidation of LDL [176]. Mouse peritoneal macrophages (MPM), used from the $PON1^{0}$ 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 $PON1^0$ mice also exhibited increased production of lipid peroxides by 84% as compared to control [176]. Furthermore, the $PON1^0$ 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 $PON1^0$ MPM that showed a 50% increase in MCB rate. Similarly, they incubated $PON1^0$ 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, HDL_3 has the highest PON1 activity and HDL_3 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 PON1Tg mice and measured the activity of PON1. The activity of PON1 was 2.4% higher in the HDL-PON1Tg 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 $U \cdot mL^{-1}$, 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 $U \cdot mL^{-1}$) 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 ($kg \cdot m^{-2}$) 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 $mg \cdot dL^{-1}$ 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 $mg \cdot dL^{-1}$ 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 $U \cdot L^{-1}$) as compared to the control (98.1 $U \cdot L^{-1}$). 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 $U \cdot L^{-1}$) than the control (274.74 $U \cdot L^{-1}$). They found that using wrestling as a form of exercise can increase PON1 and this increase was associated with a reduction in oxidative stress.

Table 1 – Summary of cross-sectional PON1 activity responses.

	Subjects	Exercise Protocol	Results
Cakmak et al. [92]. To evaluate PON1 activity and antioxidative agents between adolescent basketball players and controls.	Adolescent boys and girls	Basketball players vs. control	PON1 Activity Basketball group 177.32 ± 100.10 $U \cdot L^{-1}$ PON1 Activity Control group 98.11 ± 40.92 $U \cdot L^{-1}$ $p < 0.0001$* Intra-assay CV < 2%
Hamurcu et al. [93]. To determine the PON1 activity between adolescent wrestlers as compared to control.	Adolescent boys	Wrestlers vs controls	PON1 Activity Wrestler group 334.89 ± 124.93 $U \cdot L^{-1}$ PON1 Activity Control group 274.74 ± 87.95 $U \cdot L^{-1}$ $p = 0.024$* CV was NR

Significance, $p < 0.05$*;

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 $U \cdot L^{-1}$) as compared to before (258 $U \cdot L^{-1}$) 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% $\dot{V}O_{2max}$. 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.

Purpose	Subjects	Exercise Protocol	Results
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.	Sedentary adult men and women	16 weeks, 5 days per week for 50 minutes by swimming, running, or aerobic games	PON1 Activity before training, 258 ± 135 $U \cdot L^{-1}$ PON1 Activity after training, 253 ± 109 $U \cdot L^{-1}$ $p > 0.05$ Intra-assay CV (0.70%) Inter-assay CV (0.76%)
Richter et al. [232]. To assess whether endurance exercise which is known to be cardioprotective could beneficially influence PON1	Adult men and women with increased cardiovascular risk and coronary artery disease	12 weeks, 3-5 days per week for 40 minutes at 70 – 80% maximum HR by walking or running	PON1 Activity before training, 133 ± 6 $U \cdot L^{-1}$ PON1 Activity after training, 130 ± 5 $U \cdot L^{-1}$ $p > 0.05$ CV was NR

Goldhammer et al. [231]. To determine PON1 activity following a 12-week training program.	Adult men and women with known CAD	12 weeks, 3 days per week, 70 – 85% maximum HR on a treadmill, stationary bike, arm bicycle, rowing machine, or a combination of these activities.	PON1 Activity before training, 68.83 ± 13.51 $U \cdot L^{-1}$ PON1 Activity after training, 80.31 ± 16.22 $U \cdot L^{-1}$ $p < 0.001$ CV was NR
Casella-Filho et al. [234]. To evaluate PON1 activity in subjects with MetS before and after a 12-week training program.	20 Subjects with MetS (10 males and 10 females) 10 controls (6 men and 4 women)	12 weeks, 3 days per week, on a cycle ergometer	Significant increase in MetS group as compared to control. The author did not list results for PON1 Activity. They only had a figure to show data. $p < 0.05$ CV was NR
Rector et al. [235]. To evaluate oxidative stress following exercise training and weight loss.	21 female and 9 male subjects with MetS	4 to 7 months, 5 days per week supervised on a cycle ergometer or elliptical at an intensity of 60% $\dot{V}O_{2max}$.	PON1 Activity before training, 114.0 ± 16.8 $U \cdot L^{-1}$ PON1 Activity after training, 120.1 ± 19.3 $U \cdot L^{-1}$ $p > 0.05$ PON1 Activity, Intra-assay CV (1.9%)

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 $mL \cdot kg^{-1} \cdot min^{-1}$. 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 $U \cdot L^{-1}$) immediately after as compared to before exercise (371.43 ± 244.88 $U \cdot L^{-1}$); however, the activity returned back to baseline levels within 2 hours (388.89 ± 256.43 $U \cdot L^{-1}$).

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 $mL \cdot kg^{-1} \cdot min^{-1}$ and 26.9 ± 6.4 $mL \cdot kg^{-1} \cdot min^{-1}$. The participants

were asked to cycle for 40 minutes at 70 to 80% $\dot{V}O_{2peak.}$ 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 $mL \cdot kg^{-1} \cdot min^{-1}$ to 46.2 ± 11.0 $mL \cdot kg^{-1} \cdot min^{-1}$ 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-exericse, 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 3 - Summary of PON1 activity to acute exercise.

Purpose	Exercise Protocol	Subjects	Results
Otocha-Kmiecik et al. [95]. To evaluate the participation of plasma PON1 in antioxidant defense in response to a single bout of maximal exercise.	Maximal treadmill test Caloric expenditure NR	32 male sportsmen	PV decreases did not exceed 5%. Did not correct for PV Shifts PON1 Activity before acute bout, 371.43 ± 244.88 $U \cdot L^{-1}$ PON1 Activity after after acute bout, 436.04 ± 275.17 $U \cdot L^{-1}$ $p < 0.05$* CV was not reported.
Tomas et al. [94]. Evaluate PON1 activity, lipid levels, and oxidized LDL concentration before and after a 16-weeks aerobic exercise training period.	30minutes at maximal aerobic power on cycle ergometer before and after training Caloric expenditure NR	17 Sedentary adult men and women	PV was NR 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. $p < 0.05$* Intra-assay CV (0.70%) Inter-assay CV (0.76%)
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.	40minutes at 70 – 80% $VO_{2\ peak}$ on a cycle ergometer Caloric expenditure NR	Males and females with and without T2DM	PV was NR T2DM Group, PON1 Activity before acute bout, 109 ± 38 $U \cdot L^{-1}$ PON1 Activity after after acute bout, 106 ± 49 $U \cdot L^{-1}$ $p > 0.05$ Control Group, PON1 Activity before acute bout, 102 ± 26 $U \cdot L^{-1}$ PON1 Activity after after acute bout, 105 ± 26 $U \cdot L^{-1}$ $p > 0.05$ Inter-assay CV (8.14%)

Benitéz et al. [236]. Evaluate the immediate effects of intense aerobic exercise on the composition and oxidizability of low- (LDL) and high-density lipoproteins.	4 hour run Caloric expenditure NR	11 male athletes	Corrected for PV shifts PON1 Activity before acute bout, 157.7 ± 21.6 $U \cdot L^{-1}$ PON1 Activity after the acute bout, 153.1 ± 27.2 $U \cdot L^{-1}$ $p > 0.05$ 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% $VO_{2\ peak}$ [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 link to cardiovascular disease [238] and plays an important role in the development coronary artery disease [239]. Baldus et al. [240] found that MPO levels above the threshold of 350 $ng \cdot mL^{-1}$ 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 $\mu g \cdot mL^{-1}$) as compared to the untrained group (0.70 $\mu g \cdot mL^{-1}$). The post-exercise plasma MPO levels significantly increased as compared to rest in both the trained and untrained (1.77 $\mu g \cdot mL^{-1}$ and 1.66 $\mu g \cdot mL^{-1}$), 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% $\dot{V}O_{2max}$. 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 $\dot{V}O_{2peak}$. 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 $\dot{V}O_{2peak}$ 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 $ng \cdot mL^{-1}$ and after exercise was 24.5 $ng \cdot mL^{-1}$. The MPO levels of the exercise group before exercise was 14.7 $ng \cdot mL^{-1}$ and after exercise was 18.2 $ng \cdot mL^{-1}$. 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 $ng \cdot mL^{-1}$ and post-exercise was 20.8 $ng \cdot mL^{-1}$. 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% $\dot{V}O_{2max}$. 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 $ng \cdot mL^{-1}$ for the walking and running group. The walking group MPO concentration increased from approximately 300 $ng \cdot mL^{-1}$ to 400 $ng \cdot mL^{-1}$, which was not a significant increase following the exercise session. However, the running group's MPO level increased from approximately 300 $ng \cdot mL^{-1}$ to 600 $ng \cdot mL^{-1}$, 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 $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$. 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 $ng \cdot mL^{-1}$ from pre-exercise as compared to immediately post-exercise. However, the MPO levels for the high-intensity was significantly increased from approximately 10 $ng \cdot mL^{-1}$ to 30 $ng \cdot mL^{-1}$. 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.

Purpose	Subjects	Exercise Protocol	Results
Melanson et al. [243]. Determine MPO levels following exercise.	24 Amateur runners	Completed Boston Marathon	MPO before marathon 42.2 $ng \cdot mL^{-1}$ MPO after marathon 117.8 $ng \cdot mL^{-1}$ $p < .0001$* Inter-assay CV = 13%
Niess et al. [246]. To investigate immune response following exercise.	7 Endurance Athletes	60 minute treadmill runs at 75% $\dot{V}O_{2max}$	Results estimated from table. MPO before exercise 6 $ng \cdot mL^{-1}$ MPO after exercise 30 $ng \cdot mL^{-1}$ $p < 0.05$* CV was NR

Wetzstein et al. [238]. Evaluate the effects of an acute exercise bout on the susceptibility of isolated low density lipoprotein to in vitro oxidation.	5 Sedentary, 7 active	The sedentary group exercised on a treadmill for 30 minutes at 55% and active group exercise at 70% (exercise) of $\dot{V}O_{2peak}$.	MPO before exercise 15.8 ng·mL^{-1} MPO after exercise 20.8 ng·mL^{-1} $p < 0.05$* CV was NR
Camus et al. [247]. To compare the effects of eccentric and concentric exercises on blood nPMN and plasma levels of MPO and EL used as markers of neutrophil activation.	10 Apparently healthy males	The walking uphill and running downhill for 20 minutes at 60% $\dot{V}O_{2max}$ on a treadmill	Walking uphill $p > 0.05$ Running downhill MPO before exercise 301 ng·mL^{-1} MPO after exercise 600 ng·mL^{-1} $p < 0.05$* CV was NR
Peake et al. [245]. To investigate neutrophil degranulation at a moderate intensity and high intensity exercise.	10 well-trained male runners and triathletes	Running for 60 minutes at 60% and 85% $\dot{V}O_{2max}$ on a treadmill	60% $\dot{V}O_{2max}$. MPO did not change. $p > 0.05$ 85% $\dot{V}O_{2max}$. MPO changed from approximately 10 ng·mL^{-1} to 30 ng·mL^{-1}. $p < 0.01$* Inter-assay CV = 13.2%

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% $\dot{V}O_{2max}$. 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% $\dot{V}O_{2max}$ 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 $U \cdot L^{-1}$ to 41.1 ± 10.6 $U \cdot L^{-1}$ [94].

Table 5 - Summary of oLDL to chronic exercise.

Purpose	Subjects	Exercise Protocol	Results
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 $\mu mol \cdot L^{-1}$ Men, oLDL after training, 27.9 $\mu mol \cdot L^{-1}$ $p < 0.05$* Women, oLDL before training, 26.2 $\mu mol \cdot L^{-1}$ Women, oLDL after training, 19.4 $\mu mol \cdot L^{-1}$ $p < 0.05$* 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. $p > 0.05$

			Intra-assay CV = 5.3% Inter-assay CV = 5.2%
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 $U \cdot L^{-1}$ oLDL after training, 41.1$U \cdot L^{-1}$ $p < 0.043$* 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 $U \cdot L^{-1}$ to 54.0 $U \cdot L^{-1}$) before training. On the other hand, the oLDL was significantly increased (from 41.1 $U \cdot L^{-1}$ to 50.0 $U \cdot L^{-1}$) 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 $mg \cdot L^{-1}$ of Hermesetas® during the exercise session. The carbohydrate group would perform the same 6-hour walk, but would drink 50 $g \cdot L^{-1}$ 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 $\mu mol \cdot L^{-1}$ to 57.3 ± 2.1 $\mu mol \cdot L^{-1}$) and for trial II (from 33.6 ± 3.0 $\mu mol \cdot L^{-1}$ to 34.5 ± 2.0 $\mu mol \cdot L^{-1}$). 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 $hr \cdot wk^{-1}$ and the exercise group exercise ≥ 6 $hr \cdot wk^{-1}$. The average $\dot{V}O_{2peak}$ 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 $\dot{V}O_{2peak}$. 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 $\dot{V}O_{2max}$. 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% $\dot{V}O_{2max}$ 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.

Reference	Subjects	Exercise Protocol	Result
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.	Sedentary men and women	30 minutes at maximal aerobic power on cycle ergometer before and after 16 weeks of training	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%
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 $\mu mol \cdot L^{-1}$ and 23 $\mu mol \cdot L^{-1}$ $p < 0.01$* Carb load, NC $p > 0.05$ PRE and POST 3. oLDL, Placebo 28 $\mu mol \cdot L^{-1}$ and 21 $\mu mol \cdot L^{-1}$ $p < 0.001$* Carb load, 26 $\mu mol \cdot L^{-1}$ and 21 $\mu mol \cdot L^{-1}$ $p = 0.039$* 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 $\mu mol \cdot L^{-1}$ After marathon, 57.3 $\mu mol \cdot L^{-1}$ $p > 0.05$

			Trial II Before marathon, 33.6 $\mu mol \cdot L^{-1}$ After marathon, 34.5 $\mu mol \cdot L^{-1}$ $p > 0.05$ CV was NR
Chang et al. [257]. Investigate LDL oxidation and activities of antioxidant enzymes in WW at rest and after a rugby game.	15 Professional Rugby Players (TR) and 6 Recreational Weekend Rugby Players (WW) and 10 male Controls	Rugby Match and blood samples were taken before and after game	Baseline, TR, 19.77 $\mu mol \cdot L^{-1}$, WW, 24.66 $\mu mol \cdot L^{-1}$ vs. control, 16.07 $\mu mol \cdot L^{-1}$ $p < 0.05$* After Rugby Game, TR, 19.68 $\mu mol \cdot L^{-1}$, WW, 22.78 $\mu mol \cdot L^{-1}$ vs. control, 16.07 $\mu mol \cdot L^{-1}$ $p > 0.05$ After Rugby Game, TR, 19.68 $\mu mol \cdot L^{-1}$ vs. WW, 22.78 $\mu mol \cdot L^{-1}$ $p < 0.05$* CV was NR
Wetzstein et al. [238]. Examine the effect of an acute exercise bout on the susceptibility of isolated low density lipoprotein to in vitro oxidation.	12 Sedentary men and women. 11 Active men and women	Treadmill, 30 minutes at 55% (sedentary) and 70% (exercise) of $\dot{V}O_{2\ peak}$	Sedentary group, NC, $p > 0.05$. Exercise Group, NC, $p > 0.05$. Combined Group, A decrease in lag time is associated with increased oxidation of LDL in-vitro 96.1 to 92.1 minutes $p < 0.05$* CV was NR
Tozzi-Ciancarelli et al. [258]. To evaluate in sedentary male	15 Sedentary males	Cycle Ergometer, Maximal test and 30 minutes at	Significantly increased oLDL immediately post-exercise

subjects the effects of an acute bout of strenuous and moderate exercise on oxidative markers.		60% $\dot{V}O_{2\ max}$ 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% $\dot{V}O_{2peak}$. 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 $g \cdot mL^{-1}$ and HDL density ranges from 1.063 to 1.210 $g \cdot mL^{-1}$ [261]. The LDL is composed of Apo B100 and the reference lipid composition has a total calculated lipid composition of 775 phospholipids, 387 unesterified cholesterols, 650 cholesterol esters, and 850 TG molecules [260]. HDL is mainly composed of Apo A and the HDL_2 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 HDL_3 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% $\dot{V}O_{2\ max}$ for the first 10 weeks and then 40 minutes of aerobic exercise at 70% $\dot{V}O_{2\ max}$ 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% $\dot{V}O_{2peak}$. The LH exercise prescription would be equivalent to jogging 12 miles per week at an intensity of 65 to 80% $\dot{V}O_{2peak}$. The HH exercise prescription would be equivalent to jogging 20 miles per week at an intensity of 65 to 80% $\dot{V}O_{2peak}$. 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 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 HDL_2 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 (B_3) 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 $mg \cdot day^{-1}$. 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

[116, 290]. The decreased clearance of HDL would allow HDL size to increase from HDL_3 to HDL_2 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 $\dot{V}O_{2max}$ 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 ≥ 30 $kg \cdot m^{-2}$), hypertriglyeridemic (TG ≥ 150 $mg \cdot dL^{-1}$) 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 $mg \cdot day^{-1}$ during the first week to 1500 $mg \cdot day^{-1}$ during the third week. The 1500 $mg \cdot day^{-1}$ 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% $\dot{V}O_{2max}$. 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 $mg \cdot day^{-1}$ over a three week period. Participants were asked to begin taking 500 $mg \cdot day^{-1}$ right before bed for the first week. Dosage increased to 1000 $mg \cdot day^{-1}$ for the second week. Dosage was increased to 1500 $mg \cdot day^{-1}$ 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 Activity = [(Final Absorbance – Initial Absorbance) – Blank] x 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 $nmol \cdot L^{-1}$, 4.3 – 114.9 $nmol \cdot L^{-1}$, and 1.4 – 61.6 $nmol \cdot L^{-1}$. 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 $nmol \cdot L^{-1}$, 0 – 86 $nmol \cdot L^{-1}$, 70 – 657 $nmol \cdot L^{-1}$, 516 – 1886 $nmol \cdot L^{-1}$, 119 – 402 $nmol \cdot L^{-1}$, and 393 – 1483 $nmol \cdot L^{-1}$. 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 $\mu mol \cdot L^{-1}$, 1.6 – 10.1 $\mu mol \cdot L^{-1}$, 0 – 6.8 $\mu mol \cdot L^{-1}$, and 14.0 – 26.9 $\mu mol \cdot L^{-1}$. 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 ($kg \cdot m^{-2}$); WHR; $\dot{V}O_{2\ max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$). 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 participates maintained their body weight throughout the study [98].

Table 7a – Baseline physiological characteristics.

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

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.

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

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.

Variable	Units	Baseline	24 Post Exercise	*p* value
PON1a	$kU \cdot L^{-1}$	125.9 ± 4.9	131.5 ± 4.0	0.269
PON1c	$\mu g \cdot mL^{-1}$	112.4 ± 8.2	118.9 ± 8.5	0.391
Apo A1	$\mu g \cdot mL^{-1}$	5031.5 ± 1646.1	5052.7 ± 1325.6	0.983
Total HDL Particles	$\mu mol \cdot L^{-1}$	31.5 ± 1.3	32.1 ± 1.7	0.469
Large HDL Particles	$\mu mol \cdot L^{-1}$	3.5 ± 0.5	3.1 ± 0.5	0.068
Medium HDL Particles	$\mu mol \cdot L^{-1}$	5.1 ± 1.5	4.2 ± 1.3	0.496
Small HDL Particles	$\mu mol \cdot L^{-1}$	22.9 ± 1.4	24.9 ± 1.5	0.214
HDL Particle Size	nm	8.6 ± 0.1	8.5 ± 0.1	0.040*
HDLc	$mg \cdot dL^{-1}$	41 ± 2	40 ± 2	0.403
TG	$mg \cdot dL^{-1}$	293 ± 28	231 ± 28	0.006*

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, Triglyericide concentration.

Exercise did not alter PON1 activity ($F_{1,11} = 1.36$, $p = 0.269$) or PON1 concentration ($F_{1,11} = 0.80$, $p = 0.391$). Apo A1 was not altered ($F_{1,11} = 0.00$, $p = 0.983$). Exercise did not influence a change in the total HDL particle numbers ($F_{1,11} = 0.56$, $p = 0.469$), large HDL particle numbers ($F_{1,11} = 4.11$, $p = 0.068$), medium HDL particle numbers ($F_{1,11} = 0.50$, $p = 0.496$), and small HDL particle numbers ($F_{1,11} = 1.74$, $p = 0.214$). However, the HDL particle size was reduced significantly ($F_{1,11} = 5.39$, $p = 0.040$*). HDLc was not altered ($F_{1,11} = 0.758$, $p = 0.403$), but triglyceride was significantly decreased ($F_{1,11} = 11.40$, $p = 0.006$*) 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.

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

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.

Variable	Units	Baseline	Niacin_Pre	*p* value
PON1a	$kU \cdot L^{-1}$	125.9 ± 4.9	115.9 ± 6.2	0.143
PON1c	$\mu g \cdot mL^{-1}$	112.4 ± 8.2	105.9 ± 8.5	0.536
Apo A1	$\mu g \cdot mL^{-1}$	5031.5 ± 1646.1	4339.3 ± 1061.1	0.198
Total HDL Particles	$\mu mol \cdot L^{-1}$	31.5 ± 1.3	32.0 ± 1.1	0.775
Large HDL Particles	$\mu mol \cdot L^{-1}$	3.5 ± 0.5	3.8 ± 0.8	0.875
Medium HDL Particles	$\mu mol \cdot L^{-1}$	5.1 ± 1.5	2.9 ± 0.9	0.146
Small HDL Particles	$\mu mol \cdot L^{-1}$	22.9 ± 1.4	25.3 ± 1.1	0.313
Total HDL Particles	$\mu mol \cdot L^{-1}$	31.5 ± 1.3	32.0 ± 1.1	0.775
HDL Particle Size	nm	8.6 ± 0.1	8.6 ± 0.1	0.862
HDLc	$mg \cdot dL^{-1}$	41 ± 2	41 ± 2	0.690
TG	$mg \cdot dL^{-1}$	293 ± 28	196 ± 19	< 0.001*

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, Triglyericide concentration.

Six weeks of niacin therapy did not alter PON1 activity ($F_{1,11} = 2.49$, $p = 0.143$). Additionally, six weeks of niacin therapy did not alter PON1 concentration ($F_{1,11} = 0.41$, $p = 0.536$). Six weeks of niacin therapy did not alter Apo A1 ($F_{1,11} = 1.90$, $p = 0.198$).

Extended-release niacin had no effect on the total HDL particle numbers ($F_{1,11} = 0.09$, $p = 0.775$), large HDL particle numbers ($F_{1,11} = 0.03$, $p = 0.875$), medium HDL particle numbers ($F_{1,11} = 2.49$, $p = 0.146$), and small HDL particle numbers ($F_{1,11} = 1.13$,

p = 0.313). 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.

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

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.

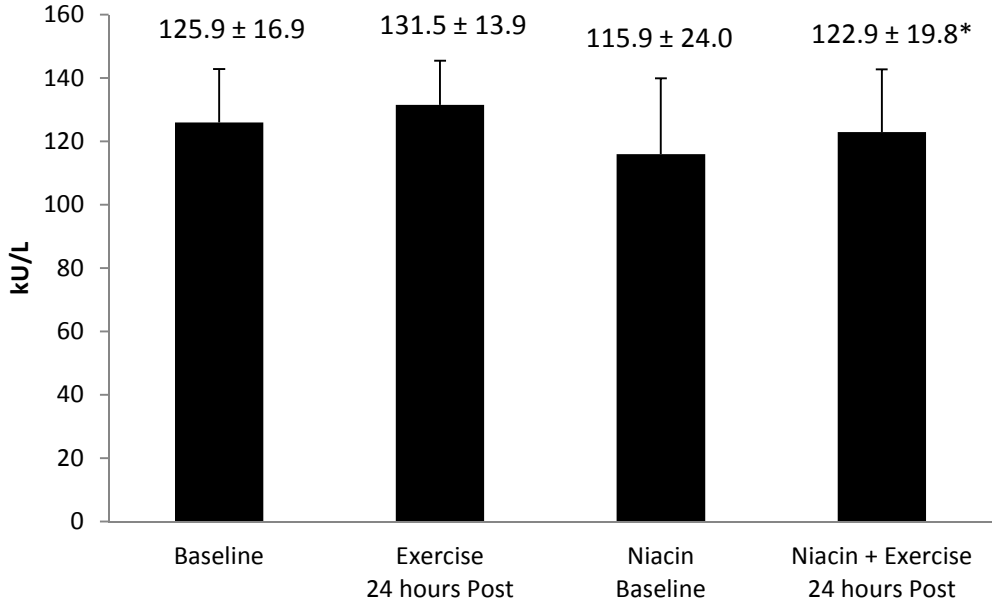
Changes in variables of interest occurring with niacin therapy (6-weeks of niacin therapy – baseline) were calculated and the relationships between baseline PON1 activity and concentration and change variables were determined. Baseline PON1 activity was not correlated with any of the change variables. 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.

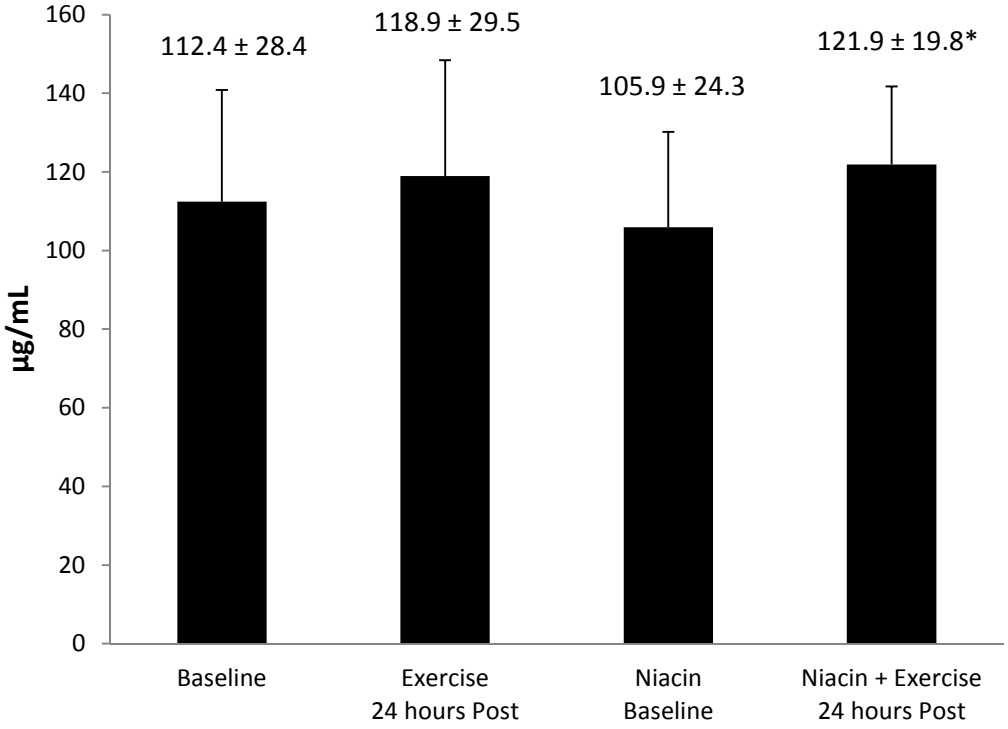
Figure 3 – PON1 activity response to exercise and niacin therapy.



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.

Variable	Units	Niacin_Pre	Niacin_AEX	*p* value
Apo A1	$\mu g \cdot mL^{-1}$	4339.3 ± 1061.1	3871.2 ± 2839.0	0.059
Total HDL Particles	$\mu mol \cdot L^{-1}$	32.0 ± 1.1	33.2 ± 1.3	0.102
Large HDL Particles	$\mu mol \cdot L^{-1}$	3.8 ± 0.8	3.8 ± 0.6	0.807
Medium HDL Particles	$\mu mol \cdot L^{-1}$	2.9 ± 0.9	3.9 ± 0.7	0.250
Small HDL Particles	$\mu mol \cdot L^{-1}$	25.3 ± 1.1	25.5 ± 1.4	0.846
HDL Particle Size	nm	8.6 ± 0.1	8.6 ± 0.1	0.831
HDLc	$mg \cdot dL^{-1}$	41 ± 2	43 ± 2	0.124
TG	$mg \cdot dL^{-1}$	196 ± 19	159 ± 12	0.006*

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, Triglyericide concentration.

Acute exercise combined with 6 weeks of niacin therapy did not alter Apo A1 ($F_{1,11} = 4.85$, $p = 0.059$). 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 ($F_{1,11} = 3.25$, $p = 0.102$). The large HDL particle numbers were not altered ($F_{1,11} = 0.06$, $p = 0.807$). The medium HDL particle numbers were not altered ($F_{1,11} = 1.49$, $p = 0.250$). The same treatments did not alter the small HDL particle numbers ($F_{1,11} = 0.04$, $p = 0.846$). HDL particle size was not altered ($F_{1,11} = 0.05$, $p = 0.831$). The combined effects of acute exercise and niacin therapy did not significantly alter HDLc ($F_{1,11} = 2.825$, $p = 0.124$). TG were significantly reduced following the combined effects of exercise and niacin therapy ($F_{1,11} = 11.70$, $p = 0.006$*).

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.

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

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 ($F_{1,11} = 2.312$, $p = 0.043$*). However, these two interventions did not alter medium VLDL particle numbers ($F_{1,11} = 0.33$, $p = 0.749$), and small VLDL particle numbers ($F_{1,11} = 1.59$, $p = 0.143$).

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

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 signficantly 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 10^{th} 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 $mg \cdot dL^{-1}$, 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 $mg \cdot day^{-1}$ 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 $mg \cdot day^{-1}$ 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 $mg \cdot day^{-1}$ 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 $mg \cdot day^{-1}$ 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 $mg \cdot day^{-1}$ 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 $mg \cdot day^{-1}$.

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-γ 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 $mg \cdot day^{-1}$ 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% $\dot{V}O_{2max}$ 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.

REFERENCES

[1] Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106(25):3143-421.

[2] Ervin RB. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States, 2003-2006. Natl Health Stat Report 2009;(13):1-7.

[3] Ford ES, Li C, Zhao G. Prevalence and correlates of metabolic syndrome based on a harmonious definition among adults in the US. J Diabetes 2010;2(3):180-93.

[4] Garin MC, Kalix B, Morabia A, et al. Small, dense lipoprotein particles and reduced paraoxonase-1 in patients with the metabolic syndrome. J Clin Endocrinol Metab 2005;90(4):2264-9.

[5] Hulthe J, Bokemark L, Wikstrand J, et al. The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (AIR) study. Arterioscler Thromb Vasc Biol 2000;20(9):2140-7.

[6] Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006;444(7121):875-80.

[7] Lamarche B, Tchernof A, Moorjani S, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997;95(1):69-75.

[8] Kontush A, de Faria EC, Chantepie S, et al. A normotriglyceridemic, low HDL-cholesterol phenotype is characterised by elevated oxidative stress and HDL particles with attenuated antioxidative activity. Atherosclerosis 2005;182(2):277-85.

[9] Kontush A, Chapman MJ. Antiatherogenic small, dense HDL--guardian angel of the arterial wall? Nat Clin Pract Cardiovasc Med 2006;3(3):144-53.

[10] Hansel B, Giral P, Nobecourt E, et al. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab 2004;89(10):4963-71.

[11] Morgan JM, Carey CM, Lincoff A, et al. The effects of niacin on lipoprotein subclass distribution. Prev Cardiol 2004;7(4):182-7; quiz 8.

[12] Yoshida H, Kisugi R. Mechanisms of LDL oxidation. Clin Chim Acta 2010;411(23-24):1875-82.

[13] Navab M, Imes SS, Hama SY, et al. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. J Clin Invest 1991;88(6):2039-46.

[14] Barter P. The inflammation: lipoprotein cycle. Atheroscler Suppl 2005;6(2):15-20.

[15] Nielsen LB. Transfer of low density lipoprotein into the arterial wall and risk of atherosclerosis. Atherosclerosis 1996;123(1-2):1-15.

[16] Liao JK, Shin WS, Lee WY, et al. Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthase. J Biol Chem 1995;270(1):319-24.

[17] Rosenson RS, Lowe GD. Effects of lipids and lipoproteins on thrombosis and rheology. Atherosclerosis 1998;140(2):271-80.
[18] Lusis AJ. Atherosclerosis. Nature 2000;407(6801):233-41.
[19] Esteve E, Ricart W, Fernandez-Real JM. Dyslipidemia and inflammation: an evolutionary conserved mechanism. Clin Nutr 2005;24(1):16-31.
[20] Bergt C, Pennathur S, Fu X, et al. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. Proc Natl Acad Sci U S A 2004;101(35):13032-7.
[21] Shao B, Heinecke JW. Impact of HDL oxidation by the myeloperoxidase system on sterol efflux by the ABCA1 pathway. J Proteomics 2011;74(11):2289-99.
[22] Diaz MN, Frei B, Vita JA, et al. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997;337(6):408-16.
[23] Rifici VA, Khachadurian AK. Oxidation of high density lipoproteins: characterization and effects on cholesterol efflux from J774 macrophages. Biochim Biophys Acta 1996;1299(1):87-94.
[24] Marsche G, Hammer A, Oskolkova O, et al. Hypochlorite-modified high density lipoprotein, a high affinity ligand to scavenger receptor class B, type I, impairs high density lipoprotein-dependent selective lipid uptake and reverse cholesterol transport. J Biol Chem 2002;277(35):32172-9.
[25] Mackness MI, Arrol S, Abbott C, et al. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis 1993;104(1-2):129-35.
[26] Aviram M, Rosenblat M, Bisgaier CL, et al. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998;101(8):1581-90.
[27] Mackness MI, Durrington PN. HDL, its enzymes and its potential to influence lipid peroxidation. Atherosclerosis 1995;115(2):243-53.
[28] Sorenson RC, Bisgaier CL, Aviram M, et al. Human serum Paraoxonase/Arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids : apolipoprotein A-I stabilizes activity. Arterioscler Thromb Vasc Biol 1999;19(9):2214-25.
[29] Watson AD, Berliner JA, Hama SY, et al. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. J Clin Invest 1995;96(6):2882-91.
[30] Khersonsky O, Tawfik DS. Structure-reactivity studies of serum paraoxonase PON1 suggest that its native activity is lactonase. Biochemistry 2005;44(16):6371-82.
[31] Aviram M, Rosenblat M. Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. Free Radic Biol Med 2004;37(9):1304-16.
[32] La Du BN. Structural and functional diversity of paraoxonases. Nat Med 1996;2(11):1186-7.
[33] Mazur A. An enzyme in animal tissues capable of hydrolysing the phosphorus-fluorine bond of alkyl fluorophosphates. J Biol Chem 1946;164:271-89.
[34] Aldridge WN. Serum esterases. I. Two types of esterase (A and B) hydrolysing p-nitrophenyl acetate, propionate and butyrate, and a method for their determination. Biochem J 1953;53(1):110-7.

[35] Aldridge WN. Serum esterases. II. An enzyme hydrolysing diethyl p-nitrophenyl phosphate (E600) and its identity with the A-esterase of mammalian sera. Biochem J 1953;53(1):117-24.
[36] Precourt LP, Amre D, Denis MC, et al. The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis 2011;214(1):20-36.
[37] Primo-Parmo SL, Sorenson RC, Teiber J, et al. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics 1996;33(3):498-507.
[38] Mackness MI, Durrington PN, Mackness B. The role of paraoxonase 1 activity in cardiovascular disease: potential for therapeutic intervention. Am J Cardiovasc Drugs 2004;4(4):211-7.
[39] Deakin S, Leviev I, Gomaraschi M, et al. Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high affinity, saturable, desorption mechanism. J Biol Chem 2002;277(6):4301-8.
[40] Deakin SP, James RW. Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. Clin Sci (Lond) 2004;107(5):435-47.
[41] Rajkovic MG, Rumora L, Barisic K. The paraoxonase 1, 2 and 3 in humans. Biochem Med (Zagreb) 2011;21(2):122-30.
[42] Fuhrman B. Regulation of hepatic paraoxonase-1 expression. J Lipids 2012;2012:684010.
[43] Draganov DI, La Du BN. Pharmacogenetics of paraoxonases: a brief review. Naunyn Schmiedebergs Arch Pharmacol 2004;369(1):78-88.
[44] Yeung DT, Josse D, Nicholson JD, et al. Structure/function analyses of human serum paraoxonase (HuPON1) mutants designed from a DFPase-like homology model. Biochim Biophys Acta 2004;1702(1):67-77.
[45] Osaki F, Ikeda Y, Suehiro T, et al. Roles of Sp1 and protein kinase C in regulation of human serum paraoxonase 1 (PON1) gene transcription in HepG2 cells. Atherosclerosis 2004;176(2):279-87.
[46] Camps J, Garcia-Heredia A, Rull A, et al. PPARs in Regulation of Paraoxonases: Control of Oxidative Stress and Inflammation Pathways. PPAR Res 2012;2012:616371.
[47] Deakin S, Leviev I, Guernier S, et al. Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element-binding protein-2. Arterioscler Thromb Vasc Biol 2003;23(11):2083-9.
[48] Leviev I, James RW. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. Arterioscler Thromb Vasc Biol 2000;20(2):516-21.
[49] Mackness B, Hine D, Liu Y, et al. Paraoxonase-1 inhibits oxidised LDL-induced MCP-1 production by endothelial cells. Biochem Biophys Res Commun 2004;318(3):680-3.
[50] Tomkin GH. Targets for intervention in dyslipidemia in diabetes. Diabetes Care 2008;31 Suppl 2:S241-8.
[51] Aviram M. HDL--associated paraoxonase 1 (PON1) and dietary antioxidants attenuate lipoprotein oxidation, macrophage foam cells formation and atherosclerosis development. Pathophysiol Haemost Thromb 2006;35(1-2):146-51.
[52] Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett 1991;286(1-2):152-4.
[53] Oram JF, Lawn RM. ABCA1. The gatekeeper for eliminating excess tissue cholesterol. J Lipid Res 2001;42(8):1173-9.

[54] Rosenblat M, Volkova N, Aviram M. Injection of paraoxonase 1 (PON1) to mice stimulates their HDL and macrophage antiatherogenicity. Biofactors 2011;37(6):462-7.
[55] Soran H, Younis NN, Charlton-Menys V, et al. Variation in paraoxonase-1 activity and atherosclerosis. Curr Opin Lipidol 2009;20(4):265-74.
[56] Ahmed Z, Babaei S, Maguire GF, et al. Paraoxonase-1 reduces monocyte chemotaxis and adhesion to endothelial cells due to oxidation of palmitoyl, linoleoyl glycerophosphorylcholine. Cardiovasc Res 2003;57(1):225-31.
[57] Rosenblat M, Volkova N, Ward J, et al. Paraoxonase 1 (PON1) inhibits monocyte-to-macrophage differentiation. Atherosclerosis 2011;219(1):49-56.
[58] Seres I, Paragh G, Deschene E, et al. Study of factors influencing the decreased HDL associated PON1 activity with aging. Exp Gerontol 2004;39(1):59-66.
[59] James RW, Leviev I, Righetti A. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease. Circulation 2000;101(19):2252-7.
[60] de Roos NM, Schouten EG, Scheek LM, et al. Replacement of dietary saturated fat with trans fat reduces serum paraoxonase activity in healthy men and women. Metabolism 2002;51(12):1534-7.
[61] Ferretti G, Bacchetti T, Moroni C, et al. Paraoxonase activity in high-density lipoproteins: a comparison between healthy and obese females. J Clin Endocrinol Metab 2005;90(3):1728-33.
[62] Mackness MI, Mackness B, Durrington PN, et al. Paraoxonase and coronary heart disease. Curr Opin Lipidol 1998;9(4):319-24.
[63] Senti, Tomas M, Anglada R, et al. Interrelationship of smoking, paraoxonase activity, and leisure time physical activity: a population-based study. Eur J Intern Med 2003;14(3):178-84.
[64] Nishio E, Watanabe Y. Cigarette smoke extract inhibits plasma paraoxonase activity by modification of the enzyme's free thiols. Biochem Biophys Res Commun 1997;236(2):289-93.
[65] Prakash M, Shetty JK, Tripathy S, et al. Serum paraoxonase in alcohol abusers associated with alcoholic liver disease. Clin Chim Acta 2007;378(1-2):232-4.
[66] Rao MN, Marmillot P, Gong M, et al. Light, but not heavy alcohol drinking, stimulates paraoxonase by upregulating liver mRNA in rats and humans. Metabolism 2003;52(10):1287-94.
[67] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444(7121):860-7.
[68] Kobayashi S, Inoue N, Ohashi Y, et al. Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. Arterioscler Thromb Vasc Biol 2003;23(8):1398-404.
[69] Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm 2010;2010.
[70] Senti M, Tomas M, Fito M, et al. Antioxidant paraoxonase 1 activity in the metabolic syndrome. J Clin Endocrinol Metab 2003;88(11):5422-6.
[71] Martinelli N, Micaglio R, Consoli L, et al. Low levels of serum paraoxonase activities are characteristic of metabolic syndrome and may influence the metabolic-syndrome-related risk of coronary artery disease. Exp Diabetes Res 2012;2012:231502.

[72] McElveen J, Mackness MI, Colley CM, et al. Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. Clin Chem 1986;32(4):671-3.
[73] Navab M, Hama-Levy S, Van Lenten BJ, et al. Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio. J Clin Invest 1997;99(8):2005-19.
[74] Ayub A, Mackness MI, Arrol S, et al. Serum paraoxonase after myocardial infarction. Arterioscler Thromb Vasc Biol 1999;19(2):330-5.
[75] Bhattacharyya T, Nicholls SJ, Topol EJ, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA 2008;299(11):1265-76.
[76] NCEP: Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285(19):2486-97.
[77] Church TS, LaMonte MJ, Barlow CE, et al. Cardiorespiratory fitness and body mass index as predictors of cardiovascular disease mortality among men with diabetes. Arch Intern Med 2005;165(18):2114-20.
[78] Drygas W, Kostka T, Jegier A, et al. Long-term effects of different physical activity levels on coronary heart disease risk factors in middle-aged men. Int J Sports Med 2000;21(4):235-41.
[79] Drygas W, Jegler A, Kunski H. Study on threshold dose of physical activity in coronary heart disease prevention. Part I. Relationship between leisure time physical activity and coronary risk factors. Int J Sports Med 1988;9(4):275-8.
[80] Kokkinos PF, Holland JC, Narayan P, et al. Miles run per week and high-density lipoprotein cholesterol levels in healthy, middle-aged men. A dose-response relationship. Arch Intern Med 1995;155(4):415-20.
[81] Crouse SF, O'Brien BC, Rohack JJ, et al. Changes in serum lipids and apolipoproteins after exercise in men with high cholesterol: influence of intensity. J Appl Physiol 1995;79(1):279-86.
[82] Grandjean PW, Crouse SF, Rohack JJ. Influence of cholesterol status on blood lipid and lipoprotein enzyme responses to aerobic exercise. J Appl Physiol 2000;89(2):472-80.
[83] Mestek ML, Garner JC, Plaisance EP, et al. Blood lipid responses after continuous and accumulated aerobic exercise. Int J Sport Nutr Exerc Metab 2006;16(3):245-54.
[84] Otocka-Kmiecik A, Orlowska-Majdak M. The role of genetic (PON1 polymorphism) and environmental factors, especially physical activity, in antioxidant function of paraoxonase. Postepy Hig Med Dosw (Online) 2009;63:668-77.
[85] Vollaard NB, Shearman JP, Cooper CE. Exercise-induced oxidative stress:myths, realities and physiological relevance. Sports Med 2005;35(12):1045-62.
[86] Rubbo H, Radi R, Trujillo M, et al. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. J Biol Chem 1994;269(42):26066-75.
[87] Stadtman ER, Levine RL. Protein oxidation. Ann N Y Acad Sci 2000;899:191-208.
[88] Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci U S A 1988;85(17):6465-7.

[89] Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochem Biophys 1993;300(2):535-43.
[90] Liu ML, Bergholm R, Makimattila S, et al. A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. Am J Physiol 1999;276(6 Pt 1):E1083-91.
[91] Sanchez-Quesada JL, Homs-Serradesanferm R, Serrat-Serrat J, et al. Increase of LDL susceptibility to oxidation occurring after intense, long duration aerobic exercise. Atherosclerosis 1995;118(2):297-305.
[92] Cakmak A, Zeyrek D, Atas A, et al. Paraoxonase activity in athletic adolescents. Pediatr Exerc Sci 2010;22(1):93-104.
[93] Hamurcu Z, Saritas N, Baskol G, et al. Effect of wrestling exercise on oxidative DNA damage, nitric oxide level and paraoxonase activity in adolescent boys. Pediatr Exerc Sci 2010;22(1):60-8.
[94] Tomas M, Elosua R, Senti M, et al. Paraoxonase1-192 polymorphism modulates the effects of regular and acute exercise on paraoxonase1 activity. J Lipid Res 2002;43(5):713-20.
[95] Otocka-Kmiecik A, Lewandowski M, Stolarek R, et al. Effect of single bout of maximal exercise on plasma antioxidant status and paraoxonase activity in young sportsmen. Redox Rep 2010;15(6):275-81.
[96] Bloomgarden ZT. Dyslipidemia and the metabolic syndrome. Diabetes Care 2004;27(12):3009-16.
[97] Pan J, Van JT, Chan E, et al. Extended-release niacin treatment of the atherogenic lipid profile and lipoprotein(a) in diabetes. Metabolism 2002;51(9):1120-7.
[98] Plaisance EP, Mestek ML, Mahurin AJ, et al. Postprandial triglyceride responses to aerobic exercise and extended-release niacin. Am J Clin Nutr 2008;88(1):30-7.
[99] Capuzzi DM, Guyton JR, Morgan JM, et al. Efficacy and safety of an extended-release niacin (Niaspan): a long-term study. Am J Cardiol 1998;82(12A):74U-81U; discussion 5U-6U.
[100] Goldberg AC. Clinical trial experience with extended-release niacin (Niaspan): dose-escalation study. Am J Cardiol 1998;82(12A):35U-8U; discussion 9U-41U.
[101] Guyton JR, Goldberg AC, Kreisberg RA, et al. Effectiveness of once-nightly dosing of extended-release niacin alone and in combination for hypercholesterolemia. Am J Cardiol 1998;82(6):737-43.
[102] Morgan JM, Capuzzi DM, Guyton JR. A new extended-release niacin (Niaspan): efficacy, tolerability, and safety in hypercholesterolemic patients. Am J Cardiol 1998;82(12A):29U-34U; discussion 9U-41U.
[103] Vosper H. Niacin: a re-emerging pharmaceutical for the treatment of dyslipidaemia. Br J Pharmacol 2009;158(2):429-41.
[104] Gille A, Bodor ET, Ahmed K, et al. Nicotinic acid: pharmacological effects and mechanisms of action. Annu Rev Pharmacol Toxicol 2008;48:79-106.
[105] Mahboubi K, Witman-Jones T, Adamus JE, et al. Triglyceride modulation by acifran analogs: activity towards the niacin high and low affinity G protein-coupled receptors HM74A and HM74. Biochem Biophys Res Commun 2006;340(2):482-90.
[106] Zhao SP, Yang J, Li J, et al. Effect of niacin on LXRalpha and PPARgamma expression and HDL-induced cholesterol efflux in adipocytes of hypercholesterolemic rabbits. Int J Cardiol 2008;124(2):172-8.

[107] Grandjean PW, Plaisance EP, Mahurin AJ, et al., editors. Fasting Blood Lipid Responses to Aerobic Exercise and Niacin Therapy in Men with Metabolic Syndrome. 54th Annual Meeting of the American College of Sports Medicine; 2007; New Orleans, LA.
[108] Grandjean PW, Plaisance EP, Mahurin AJ, et al., editors. Aerobic Exercise and Niacin Therapy Alter Lipoprotein Characteristics in Metabolic Syndrome. 56th Annual Meeting of the American College of Sports Medicine; 2009; Seattle, WA.
[109] Goldberg A, Alagona P, Jr., Capuzzi DM, et al. Multiple-dose efficacy and safety of an extended-release form of niacin in the management of hyperlipidemia. Am J Cardiol 2000;85(9):1100-5.
[110] Knopp RH, Alagona P, Davidson M, et al. Equivalent efficacy of a time-release form of niacin (Niaspan) given once-a-night versus plain niacin in the management of hyperlipidemia. Metabolism 1998;47(9):1097-104.
[111] Superko RH. Lipoprotein subclasses and atherosclerosis. Front Biosci 2001;6:D355-65.
[112] Desai K, Bruckdorfer KR, Hutton RA, et al. Binding of apoE-rich high density lipoprotein particles by saturable sites on human blood platelets inhibits agonist-induced platelet aggregation. J Lipid Res 1989;30(6):831-40.
[113] Eisenberg S. High density lipoprotein metabolism. J Lipid Res 1984;25(10):1017-58.
[114] Sorrentino SA, Besler C, Rohrer L, et al. Endothelial-vasoprotective effects of high-density lipoprotein are impaired in patients with type 2 diabetes mellitus but are improved after extended-release niacin therapy. Circulation 2010;121(1):110-22.
[115] Blaha MJ, Bansal S, Rouf R, et al. A practical "ABCDE" approach to the metabolic syndrome. Mayo Clin Proc 2008;83(8):932-41.
[116] Kamanna VS, Kashyap ML. Mechanism of action of niacin on lipoprotein metabolism. Curr Atheroscler Rep 2000;2(1):36-46.
[117] Linke A, Sonnabend M, Fasshauer M, et al. Effects of extended-release niacin on lipid profile and adipocyte biology in patients with impaired glucose tolerance. Atherosclerosis 2009;205(1):207-13.
[118] Aviram M, Rosenblat M. Paraoxonases and cardiovascular diseases: pharmacological and nutritional influences. Curr Opin Lipidol 2005;16(4):393-9.
[119] Grip O, Janciauskiene S, Lindgren S. Atorvastatin activates PPAR-gamma and attenuates the inflammatory response in human monocytes. Inflamm Res 2002;51(2):58-62.
[120] Shen Y, Wu H, Wang C, et al. Simvastatin attenuates cardiopulmonary bypass-induced myocardial inflammatory injury in rats by activating peroxisome proliferator-activated receptor gamma. Eur J Pharmacol 2010;649(1-3):255-62.
[121] Tomas M, Senti M, Garcia-Faria F, et al. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2000;20(9):2113-9.
[122] Knowles HJ, te Poele RH, Workman P, et al. Niacin induces PPARgamma expression and transcriptional activation in macrophages via HM74 and HM74a-mediated induction of prostaglandin synthesis pathways. Biochem Pharmacol 2006;71(5):646-56.
[123] Durstine JL, Grandjean PW, Davis PG, et al. Blood lipid and lipoprotein adaptations to exercise: a quantitative analysis. Sports Med 2001;31(15):1033-62.

[124] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15(7):539-53.
[125] Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med 2006;23(5):469-80.
[126] Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112(17):2735-52.
[127] Ford ES, Schulze MB, Pischon T, et al. Metabolic syndrome and risk of incident diabetes: findings from the European Prospective Investigation into Cancer and Nutrition-Potsdam Study. Cardiovasc Diabetol 2008;7:35.
[128] Laaksonen DE, Lakka HM, Salonen JT, et al. Low levels of leisure-time physical activity and cardiorespiratory fitness predict development of the metabolic syndrome. Diabetes Care 2002;25(9):1612-8.
[129] Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 2002;287(3):356-9.
[130] Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol 2008;28(4):629-36.
[131] Carr DB, Utzschneider KM, Hull RL, et al. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. Diabetes 2004;53(8):2087-94.
[132] Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. Annu Rev Nutr 2005;25:391-406.
[133] Reaven G. All obese individuals are not created equal: insulin resistance is the major determinant of cardiovascular disease in overweight/obese individuals. Diab Vasc Dis Res 2005;2(3):105-12.
[134] Flegal KM, Carroll MD, Kit BK, et al. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. JAMA 2012;307(5):491-7.
[135] Yanovski SZ. Overweight, obesity, and health risk: National Task Force on the Prevention and Treatment of Obesity. Arch Intern Med 2000;160(7):898-904.
[136] Field AE, Coakley EH, Must A, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch Intern Med 2001;161(13):1581-6.
[137] Chan JM, Rimm EB, Colditz GA, et al. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care 1994;17(9):961-9.
[138] Colditz GA, Willett WC, Rotnitzky A, et al. Weight gain as a risk factor for clinical diabetes mellitus in women. Ann Intern Med 1995;122(7):481-6.
[139] Vinik AI. The metabolic basis of atherogenic dyslipidemia. Clin Cornerstone 2005;7(2-3):27-35.
[140] Kuk JL, Church TS, Blair SN, et al. Does measurement site for visceral and abdominal subcutaneous adipose tissue alter associations with the metabolic syndrome? Diabetes Care 2006;29(3):679-84.
[141] Kuk JL, Katzmarzyk PT, Nichaman MZ, et al. Visceral fat is an independent predictor of all-cause mortality in men. Obesity (Silver Spring) 2006;14(2):336-41.
[142] Arner E, Westermark PO, Spalding KL, et al. Adipocyte turnover: relevance to human adipose tissue morphology. Diabetes 2010;59(1):105-9.

[143] Bastard JP, Maachi M, Lagathu C, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 2006;17(1):4-12.
[144] Dahm CC, Gorst-Rasmussen A, Jakobsen MU, et al. Adipose tissue fatty acid patterns and changes in anthropometry: a cohort study. PLoS One 2011;6(7):e22587.
[145] Sorensen K, Aksglaede L, Munch-Andersen T, et al. Sex hormone-binding globulin levels predict insulin sensitivity, disposition index, and cardiovascular risk during puberty. Diabetes Care 2009;32(5):909-14.
[146] McLaughlin T, Allison G, Abbasi F, et al. Prevalence of insulin resistance and associated cardiovascular disease risk factors among normal weight, overweight, and obese individuals. Metabolism 2004;53(4):495-9.
[147] McLaughlin T, Abbasi F, Cheal K, et al. Use of metabolic markers to identify overweight individuals who are insulin resistant. Ann Intern Med 2003;139(10):802-9.
[148] Stolar M. Metabolic syndrome: controversial but useful. Cleve Clin J Med 2007;74(3):199-202, 5-8.
[149] Masharani UB, Maddux BA, Li X, et al. Insulin resistance in non-obese subjects is associated with activation of the JNK pathway and impaired insulin signaling in skeletal muscle. PLoS One 2011;6(5):e19878.
[150] Facchini FS, Hua N, Abbasi F, et al. Insulin resistance as a predictor of age-related diseases. J Clin Endocrinol Metab 2001;86(8):3574-8.
[151] Bloomgarden ZT. Definitions of the insulin resistance syndrome: the 1st World Congress on the Insulin Resistance Syndrome. Diabetes Care 2004;27(3):824-30.
[152] Heart Disease and Stroke Statistics - 2005 Update [database on the Internet]. 2005. Available from: http://www.americanheart.org.
[153] Goff DC, Jr., Bertoni AG, Kramer H, et al. Dyslipidemia prevalence, treatment, and control in the Multi-Ethnic Study of Atherosclerosis (MESA): gender, ethnicity, and coronary artery calcium. Circulation 2006;113(5):647-56.
[154] Tabenkin H, Eaton CB, Roberts MB, et al. Differences in cardiovascular disease risk factor management in primary care by sex of physician and patient. Ann Fam Med 2010;8(1):25-32.
[155] Bays H, Abate N, Chandalia M. Adiposopathy: sick fat causes high blood sugar, high blood pressure and dyslipidemia. Future Cardiol 2005;1(1):39-59.
[156] Adeli K, Taghibiglou C, Van Iderstine SC, et al. Mechanisms of hepatic very low-density lipoprotein overproduction in insulin resistance. Trends Cardiovasc Med 2001;11(5):170-6.
[157] Bastard JP, Pieroni L, Hainque B. Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. Diabetes Metab Res Rev 2000;16(3):192-201.
[158] Daly PA, Landsberg L. Hypertension in obesity and NIDDM. Role of insulin and sympathetic nervous system. Diabetes Care 1991;14(3):240-8.
[159] Eckel RH, Yost TJ, Jensen DR. Alterations in lipoprotein lipase in insulin resistance. Int J Obes Relat Metab Disord 1995;19 Suppl 1:S16-21.
[160] Choi SH, Ginsberg HN. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. Trends Endocrinol Metab 2011;22(9):353-63.
[161] Marcel YL, Hogue M, Weech PK, et al. Expression of apolipoprotein B epitopes in lipoproteins. Relationship to conformation and function. Arteriosclerosis 1988;8(6):832-44.

[162] Austin MA, King MC, Vranizan KM, et al. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 1990;82(2):495-506.
[163] Quinn MT, Parthasarathy S, Fong LG, et al. Oxidatively modified low density lipoprotein: A potential role in recruitment and retention of monocyte/macrophages in atherogenesis. Proc Natl Acad Sci U S A 1987;84:2995.
[164] Murakami T, Michelagnoli S, Longhi R, et al. Triglycerides are major determinants of cholesterol esterification/transfer and HDL remodeling in human plasma. Arterioscler Thromb Vasc Biol 1995;15(11):1819-28.
[165] Huang Y, von Eckardstein A, Wu S, et al. Cholesterol efflux, cholesterol esterification, and cholesteryl ester transfer by LpA-I and LpA-I/A-II in native plasma. Arterioscler Thromb Vasc Biol 1995;15(9):1412-8.
[166] Gordon T, Castelli WP, Hjortland MC, et al. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977;62(5):707-14.
[167] Shao B, Oda MN, Oram JF, et al. Myeloperoxidase: an oxidative pathway for generating dysfunctional high-density lipoprotein. Chem Res Toxicol 2010;23(3):447-54.
[168] Getz GS, Reardon CA. Paraoxonase, a cardioprotective enzyme: continuing issues. Curr Opin Lipidol 2004;15(3):261-7.
[169] Harel M, Aharoni A, Gaidukov L, et al. Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotic enzymes. Nat Struct Mol Biol 2004;11(5):412-9.
[170] Boman H. Cholinesterase, arylesterase, and lipoprotein parameters in twins. Acta Genet Med Gemellol (Roma) 1980;29(4):281-7.
[171] Acton S, Rigotti A, Landschulz KT, et al. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science 1996;271(5248):518-20.
[172] Gaidukov L, Tawfik DS. High affinity, stability, and lactonase activity of serum paraoxonase PON1 anchored on HDL with ApoA-I. Biochemistry 2005;44(35):11843-54.
[173] Gaidukov L, Viji RI, Yacobson S, et al. ApoE induces serum paraoxonase PON1 activity and stability similar to ApoA-I. Biochemistry 2010;49(3):532-8.
[174] Shih DM, Gu L, Xia YR, et al. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 1998;394(6690):284-7.
[175] Ng DS, Chu T, Esposito B, et al. Paraoxonase-1 deficiency in mice predisposes to vascular inflammation, oxidative stress, and thrombogenicity in the absence of hyperlipidemia. Cardiovasc Pathol 2008;17(4):226-32.
[176] Rozenberg O, Rosenblat M, Coleman R, et al. Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice. Free Radic Biol Med 2003;34(6):774-84.
[177] Rosenblat M, Aviram M. Oxysterol-induced activation of macrophage NADPH-oxidase enhances cell-mediated oxidation of LDL in the atherosclerotic apolipoprotein E deficient mouse: inhibitory role for vitamin E. Atherosclerosis 2002;160(1):69-80.
[178] Aviram M, Rosenblat M, Billecke S, et al. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic Biol Med 1999;26(7-8):892-904.
[179] Tward A, Xia YR, Wang XP, et al. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. Circulation 2002;106(4):484-90.

[180] Zhao Y, Ma Y, Fang Y, et al. Association between PON1 activity and coronary heart disease risk: a meta-analysis based on 43 studies. Mol Genet Metab 2012;105(1):141-8.
[181] Cohen J. Statistical power analysis for the behavioral sciences. New York,: Academic Press; 1969.
[182] Wang M, Lang X, Cui S, et al. Quantitative assessment of the influence of paraoxonase 1 activity and coronary heart disease risk. DNA Cell Biol 2012;31(6):975-82.
[183] Mackness B, Durrington P, McElduff P, et al. Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. Circulation 2003;107(22):2775-9.
[184] Ikeda Y, Inoue M, Suehiro T, et al. Low human paraoxonase predicts cardiovascular events in Japanese patients with type 2 diabetes. Acta Diabetol 2009;46(3):239-42.
[185] Suske G. The Sp-family of transcription factors. Gene 1999;238(2):291-300.
[186] Li L, He S, Sun JM, et al. Gene regulation by Sp1 and Sp3. Biochem Cell Biol 2004;82(4):460-71.
[187] Ikeda Y, Suehiro T, Arii K, et al. High glucose induces transactivation of the human paraoxonase 1 gene in hepatocytes. Metabolism 2008;57(12):1725-32.
[188] Semple RK, Chatterjee VK, O'Rahilly S. PPAR gamma and human metabolic disease. J Clin Invest 2006;116(3):581-9.
[189] Tontonoz P, Hu E, Graves RA, et al. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 1994;8(10):1224-34.
[190] Sears IB, MacGinnitie MA, Kovacs LG, et al. Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. Mol Cell Biol 1996;16(7):3410-9.
[191] Marx N, Sukhova G, Murphy C, et al. Macrophages in human atheroma contain PPARgamma: differentiation-dependent peroxisomal proliferator-activated receptor gamma(PPARgamma) expression and reduction of MMP-9 activity through PPARgamma activation in mononuclear phagocytes in vitro. Am J Pathol 1998;153(1):17-23.
[192] Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 1994;79(7):1147-56.
[193] Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998;391(6662):82-6.
[194] Picard F, Auwerx J. PPAR(gamma) and glucose homeostasis. Annu Rev Nutr 2002;22:167-97.
[195] Ricote M, Huang J, Fajas L, et al. Expression of the peroxisome proliferator-activated receptor gamma (PPARgamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc Natl Acad Sci U S A 1998;95(13):7614-9.
[196] Tenenbaum A, Motro M, Fisman EZ. Dual and pan-peroxisome proliferator-activated receptors (PPAR) co-agonism: the bezafibrate lessons. Cardiovasc Diabetol 2005;4:14.
[197] Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. Annu Rev Biochem 2008;77:289-312.
[198] Shimano H, Horton JD, Hammer RE, et al. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J Clin Invest 1996;98(7):1575-84.

[199] Horton JD, Shimomura I, Brown MS, et al. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. J Clin Invest 1998;101(11):2331-9.
[200] Wong J, Quinn CM, Brown AJ. SREBP-2 positively regulates transcription of the cholesterol efflux gene, ABCA1, by generating oxysterol ligands for LXR. Biochem J 2006;400(3):485-91.
[201] Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. Cell 2006;124(1):35-46.
[202] Blatter Garin MC, Abbott C, Messmer S, et al. Quantification of human serum paraoxonase by enzyme-linked immunoassay: population differences in protein concentrations. Biochem J 1994;304:549-54.
[203] Rozenberg O, Shih DM, Aviram M. Human serum paraoxonase 1 decreases macrophage cholesterol biosynthesis: possible role for its phospholipase-A2-like activity and lysophosphatidylcholine formation. Arterioscler Thromb Vasc Biol 2003;23(3):461-7.
[204] Sato R, Goldstein JL, Brown MS. Replacement of serine-871 of hamster 3-hydroxy-3-methylglutaryl-CoA reductase prevents phosphorylation by AMP-activated kinase and blocks inhibition of sterol synthesis induced by ATP depletion. Proc Natl Acad Sci U S A 1993;90(20):9261-5.
[205] Koh KK. Effects of statins on vascular wall: vasomotor function, inflammation, and plaque stability. Cardiovasc Res 2000;47(4):648-57.
[206] Rosenblat M, Vaya J, Shih D, et al. Paraoxonase 1 (PON1) enhances HDL-mediated macrophage cholesterol efflux via the ABCA1 transporter in association with increased HDL binding to the cells: a possible role for lysophosphatidylcholine. Atherosclerosis 2005;179(1):69-77.
[207] Berrougui H, Loued S, Khalil A. Purified human paraoxonase-1 interacts with plasma membrane lipid rafts and mediates cholesterol efflux from macrophages. Free Radic Biol Med 2012.
[208] Aviram M. Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. Free Radic Res 2000;33 Suppl:S85-97.
[209] Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989;320(14):915-24.
[210] Schultz D, Harrison DG. Quest for fire: seeking the source of pathogenic oxygen radicals in atherosclerosis. Arterioscler Thromb Vasc Biol 2000;20(6):1412-3.
[211] Bergmeier C, Siekmeier R, Gross W. Distribution spectrum of paraoxonase activity in HDL fractions. Clin Chem 2004;50(12):2309-15.
[212] Rosenblat M, Karry R, Aviram M. Paraoxonase 1 (PON1) is a more potent antioxidant and stimulant of macrophage cholesterol efflux, when present in HDL than in lipoprotein-deficient serum: relevance to diabetes. Atherosclerosis 2006;187(1):74-81.
[213] Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. Circulation 2013;127(1):e6-e245.
[214] Haskell WL, Lee IM, Pate RR, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Circulation 2007;116(9):1081-93.
[215] Adult participation in recommended levels of physical activity--United States, 2001 and 2003. MMWR Morb Mortal Wkly Rep 2005;54(47):1208-12.

[216] Miller NE, Rao S, Lewis B, et al. High-density lipoprotein and physical activity. Lancet 1979;1(8107):111.
[217] Nakamura N, Uzawa H, Maeda H, et al. Physical fitness. Its contribution to serum high density lipoprotein. Atherosclerosis 1983;48(2):173-83.
[218] Dufaux B, Order U, Muller R, et al. Delayed effects of prolonged exercise on serum lipoproteins. Metabolism 1986;35(2):105-9.
[219] Kantor MA, Cullinane EM, Herbert PN, et al. Acute increase in lipoprotein lipase following prolonged exercise. Metabolism 1984;33(5):454-7.
[220] Halverstadt A, Phares DA, Wilund KR, et al. Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. Metabolism 2007;56(4):444-50.
[221] LaMonte MJ, Barlow CE, Jurca R, et al. Cardiorespiratory fitness is inversely associated with the incidence of metabolic syndrome: a prospective study of men and women. Circulation 2005;112(4):505-12.
[222] Ford ES, Kohl HW, 3rd, Mokdad AH, et al. Sedentary behavior, physical activity, and the metabolic syndrome among U.S. adults. Obes Res 2005;13(3):608-14.
[223] Irwin ML, Ainsworth BE, Mayer-Davis EJ, et al. Physical activity and the metabolic syndrome in a tri-ethnic sample of women. Obes Res 2002;10(10):1030-7.
[224] Lakka TA, Laaksonen DE, Lakka HM, et al. Sedentary lifestyle, poor cardiorespiratory fitness, and the metabolic syndrome. Med Sci Sports Exerc 2003;35(8):1279-86.
[225] Sawada SS, Lee IM, Muto T, et al. Cardiorespiratory fitness and the incidence of type 2 diabetes: prospective study of Japanese men. Diabetes Care 2003;26(10):2918-22.
[226] Shaper AG, Wannamethee SG, Walker M. Body weight: implications for the prevention of coronary heart disease, stroke, and diabetes mellitus in a cohort study of middle aged men. BMJ 1997;314(7090):1311-7.
[227] Weinstein AR, Sesso HD, Lee IM, et al. Relationship of physical activity vs body mass index with type 2 diabetes in women. JAMA 2004;292(10):1188-94.
[228] Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344(18):1343-50.
[229] Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346(6):393-403.
[230] Katzmarzyk PT, Leon AS, Wilmore JH, et al. Targeting the metabolic syndrome with exercise: evidence from the HERITAGE Family Study. Med Sci Sports Exerc 2003;35(10):1703-9.
[231] Goldhammer E, Ben-Sira D, Zaid G, et al. Paraoxonase activity following exercise-based cardiac rehabilitation program. J Cardiopulm Rehabil Prev 2007;27(3):151-4.
[232] Richter B, Niessner A, Penka M, et al. Endurance training reduces circulating asymmetric dimethylarginine and myeloperoxidase levels in persons at risk of coronary events. Thromb Haemost 2005;94(6):1306-11.
[233] Aviram M, Hardak E, Vaya J, et al. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. Circulation 2000;101(21):2510-7.

[234] Casella-Filho A, Chagas AC, Maranhao RC, et al. Effect of exercise training on plasma levels and functional properties of high-density lipoprotein cholesterol in the metabolic syndrome. Am J Cardiol 2011;107(8):1168-72.
[235] Rector RS, Warner SO, Liu Y, et al. Exercise and diet induced weight loss improves measures of oxidative stress and insulin sensitivity in adults with characteristics of the metabolic syndrome. Am J Physiol Endocrinol Metab 2007;293(2):E500-6.
[236] Benitez S, Sanchez-Quesada JL, Lucero L, et al. Changes in low-density lipoprotein electronegativity and oxidizability after aerobic exercise are related to the increase in associated non-esterified fatty acids. Atherosclerosis 2002;160(1):223-32.
[237] Iborra RT, Ribeiro IC, Neves MQ, et al. Aerobic exercise training improves the role of high-density lipoprotein antioxidant and reduces plasma lipid peroxidation in type 2 diabetes mellitus. Scand J Med Sci Sports 2008;18(6):742-50.
[238] Wetzstein CJ, Shern-Brewer RA, Santanam N, et al. Does acute exercise affect the susceptibility of low density lipoprotein to oxidation? Free Radic Biol Med 1998;24(4):679-82.
[239] Zhang R, Brennan ML, Fu X, et al. Association between myeloperoxidase levels and risk of coronary artery disease. JAMA 2001;286(17):2136-42.
[240] Baldus S, Heeschen C, Meinertz T, et al. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 2003;108(12):1440-5.
[241] Butterfield TA, Best TM, Merrick MA. The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair. J Athl Train 2006;41(4):457-65.
[242] Morozov VI, Tsyplenkov PV, Golberg ND, et al. The effects of high-intensity exercise on skeletal muscle neutrophil myeloperoxidase in untrained and trained rats. Eur J Appl Physiol 2006;97(6):716-22.
[243] Melanson SE, Green SM, Wood MJ, et al. Elevation of myeloperoxidase in conjunction with cardiac-specific markers after marathon running. Am J Clin Pathol 2006;126(6):888-93.
[244] Camus G, Nys M, Poortmans JR, et al. Possible in vivo tolerance of human polymorphonuclear neutrophil to low-grade exercise-induced endotoxaemia. Mediators Inflamm 1998;7(6):413-5.
[245] Peake J, Wilson G, Hordern M, et al. Changes in neutrophil surface receptor expression, degranulation, and respiratory burst activity after moderate- and high-intensity exercise. J Appl Physiol 2004;97(2):612-8.
[246] Niess AM, Fehrenbach E, Lehmann R, et al. Impact of elevated ambient temperatures on the acute immune response to intensive endurance exercise. Eur J Appl Physiol 2003;89(3-4):344-51.
[247] Camus G, Pincemail J, Ledent M, et al. Plasma levels of polymorphonuclear elastase and myeloperoxidase after uphill walking and downhill running at similar energy cost. Int J Sports Med 1992;13(6):443-6.
[248] Szostak J, Laurant P. The forgotten face of regular physical exercise: a 'natural' anti-atherogenic activity. Clin Sci (Lond) 2011;121(3):91-106.
[249] Fisher-Wellman KH, Neufer PD. Linking mitochondrial bioenergetics to insulin resistance via redox biology. Trends Endocrinol Metab 2012;23(3):142-53.
[250] Steinberg D. Lewis A. Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. Circulation 1997;95(4):1062-71.
[251] Vasankari TJ, Kujala UM, Vasankari TM, et al. Reduced oxidized LDL levels after a 10-month exercise program. Med Sci Sports Exerc 1998;30(10):1496-501.

[252] Blache D, Lussier-Cacan S, Gagnon J, et al. Effect of exercise training on in vitro LDL oxidation and free radical-induced hemolysis: the HERITAGE Family Study. Antioxid Redox Signal 2007;9(1):123-30.
[253] Wang JS, Lin CC, Chen JK, et al. Role of chronic exercise in decreasing oxidized LDL-potentiated platelet activation by enhancing platelet-derived no release and bioactivity in rats. Life Sci 2000;66(20):1937-48.
[254] Vasankari TJ, Kujala UM, Vasankari TM, et al. Effects of acute prolonged exercise on-serum and LDL oxidation and antioxidant defences. Free Radic Biol Med 1997;22(3):509-13.
[255] Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. Dyn Med 2009;8:1.
[256] Vuorimaa T, Ahotupa M, Irjala K, et al. Acute prolonged exercise reduces moderately oxidized LDL in healthy men. Int J Sports Med 2005;26(6):420-5.
[257] Chang CK, Tseng HF, Hsuuw YD, et al. Higher LDL oxidation at rest and after a rugby game in weekend warriors. Ann Nutr Metab 2002;46(3-4):103-7.
[258] Tozzi-Ciancarelli MG, Penco M, Di Massimo C. Influence of acute exercise on human platelet responsiveness: possible involvement of exercise-induced oxidative stress. Eur J Appl Physiol 2002;86(3):266-72.
[259] Ahotupa M, Ruutu M, Mantyla E. Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. Clin Biochem 1996;29(2):139-44.
[260] Shen MM, Krauss RM, Lindgren FT, et al. Heterogeneity of serum low density lipoproteins in normal human subjects. J Lipid Res 1981;22(2):236-44.
[261] Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 2002;43(9):1363-79.
[262] Shen BW, Scanu AM, Kezdy FJ. Structure of human serum lipoproteins inferred from compositional analysis. Proc Natl Acad Sci U S A 1977;74(3):837-41.
[263] Jonas A, Phillips MC. Lipoprotein structure. In: Vance DE, Vance JE, editors. Biochemistry of lipids, lipoproteins and membranes. 5th ed. Amsterdam ; Boston: Elsevier; 2008. p. 485-506.
[264] Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982;23(1):97-104.
[265] Nichols AV, Krauss RM, Musliner TA. Nondenaturing polyacrylamide gradient gel electrophoresis. Methods Enzymol 1986;128:417-31.
[266] Blanche PJ, Gong EL, Forte TM, et al. Characterization of human high-density lipoproteins by gradient gel electrophoresis. Biochim Biophys Acta 1981;665(3):408-19.
[267] Otvos JD, Jeyarajah EJ, Bennett DW. Quantification of plasma lipoproteins by proton nuclear magnetic resonance spectroscopy. Clin Chem 1991;37(3):377-86.
[268] Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. Clin Lab Med 2006;26(4):847-70.
[269] Otvos JD, Jeyarajah EJ, Bennett DW, et al. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. Clin Chem 1992;38(9):1632-8.
[270] Carmena R, Duriez P, Fruchart JC. Atherogenic lipoprotein particles in atherosclerosis. Circulation 2004;109(23 Suppl 1):III2-7.
[271] Kamigaki AS, Siscovick DS, Schwartz SM, et al. Low density lipoprotein particle size and risk of early-onset myocardial infarction in women. Am J Epidemiol 2001;153(10):939-45.

[272] Pascot A, Lemieux I, Prud'homme D, et al. Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity. J Lipid Res 2001;42(12):2007-14.
[273] Chapman MJ, Guerin M, Bruckert E. Atherogenic, dense low-density lipoproteins. Pathophysiology and new therapeutic approaches. Eur Heart J 1998;19 Suppl A:A24-30.
[274] Cromwell WC, Otvos JD. Low-density lipoprotein particle number and risk for cardiovascular disease. Curr Atheroscler Rep 2004;6(5):381-7.
[275] Kraus WE, Houmard JA, Duscha BD, et al. Effects of the amount and intensity of exercise on plasma lipoproteins. N Engl J Med 2002;347(19):1483-92.
[276] Kantor MA, Cullinane EM, Sady SP, et al. Exercise acutely increases high density lipoprotein-cholesterol and lipoprotein lipase activity in trained and untrained men. Metabolism 1987;36(2):188-92.
[277] Harrison M, Moyna NM, Zderic TW, et al. Lipoprotein particle distribution and skeletal muscle lipoprotein lipase activity after acute exercise. Lipids Health Dis 2012;11:64.
[278] Grundy SM, Vega GL, McGovern ME, et al. Efficacy, safety, and tolerability of once-daily niacin for the treatment of dyslipidemia associated with type 2 diabetes: results of the assessment of diabetes control and evaluation of the efficacy of niaspan trial. Arch Intern Med 2002;162(14):1568-76.
[279] Elam MB, Hunninghake DB, Davis KB, et al. Effect of niacin on lipid and lipoprotein levels and glycemic control in patients with diabetes and peripheral arterial disease: the ADMIT study: A randomized trial. Arterial Disease Multiple Intervention Trial. JAMA 2000;284(10):1263-70.
[280] Tavintharan S, Kashyap ML. The benefits of niacin in atherosclerosis. Curr Atheroscler Rep 2001;3(1):74-82.
[281] Hopkins GJ, Barter PJ. Role of triglyceride-rich lipoproteins and hepatic lipase in determining the particle size and composition of high density lipoproteins. J Lipid Res 1986;27(12):1265-77.
[282] Patsch JR, Prasad S, Gotto AM, Jr., et al. Postprandial lipemia. A key for the conversion of high density lipoprotein2 into high density lipoprotein3 by hepatic lipase. J Clin Invest 1984;74(6):2017-23.
[283] Kuvin JT, Dave DM, Sliney KA, et al. Effects of extended-release niacin on lipoprotein particle size, distribution, and inflammatory markers in patients with coronary artery disease. Am J Cardiol 2006;98(6):743-5.
[284] Ganji SH, Tavintharan S, Zhu D, et al. Niacin noncompetitively inhibits DGAT2 but not DGAT1 activity in HepG2 cells. J Lipid Res 2004;45(10):1835-45.
[285] Carlson LA. Studies on the effect of nicotinic acid on catecholamine stimulated lipolysis in adipose tissue in vitro. Acta Med Scand 1963;173:719-22.
[286] Hughes TA, Stentz F, Gettys T, et al. Combining beta-adrenergic and peroxisome proliferator-activated receptor gamma stimulation improves lipoprotein composition in healthy moderately obese subjects. Metabolism 2006;55(1):26-34.
[287] Herrera E, Lasuncion MA, Gomez-Coronado D, et al. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. Am J Obstet Gynecol 1988;158(6 Pt 2):1575-83.
[288] Wise A, Foord SM, Fraser NJ, et al. Molecular identification of high and low affinity receptors for nicotinic acid. J Biol Chem 2003;278(11):9869-74.
[289] Wanders D, Judd RL. Future of GPR109A agonists in the treatment of dyslipidaemia. Diabetes Obes Metab 2011;13(8):685-91.

[290] Kamanna VS, Kashyap ML. Mechanism of action of niacin. Am J Cardiol 2008;101(8A):20B-6B.
[291] Costa LG, Kelada SN, Costa-Mallen P, et al. Paraoxonase 2 (PON2) Polymorphisms and Parkinson's Disease. Neuroscience Research Communications 2004;34(3):130-5.
[292] Draganov DI, Stetson PL, Watson CE, et al. Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. J Biol Chem 2000;275(43):33435-42.
[293] Ng CJ, Wadleigh DJ, Gangopadhyay A, et al. Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low density lipoprotein. J Biol Chem 2001;276(48):44444-9.
[294] Sampietro T, Bigazzi F, Dal Pino B, et al. HDL: the 'new' target of cardiovascular medicine. Int J Cardiol 2006;108(2):143-54.
[295] Thompson WR, Gordon NF, Pescatello LS, editors. ACSM's guidelines for exercise testing and prescription. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2010.
[296] Executive summary of the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. Arch Intern Med 1998;158(17):1855-67.
[297] Nielsen JN, Frosig C, Sajan MP, et al. Increased atypical PKC activity in endurance-trained human skeletal muscle. Biochem Biophys Res Commun 2003;312(4):1147-53.
[298] Taylor AJ, Sullenberger LE, Lee HJ, et al. Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. Circulation 2004;110(23):3512-7.
[299] Prado KB, Shugg S, Backstrand JR. Low-density lipoprotein particle number predicts coronary artery calcification in asymptomatic adults at intermediate risk of cardiovascular disease. J Clin Lipidol 2011;5(5):408-13.
[300] Jafri H, Alsheikh-Ali AA, Mooney P, et al. Extended-release niacin reduces LDL particle number without changing total LDL cholesterol in patients with stable CAD. J Clin Lipidol 2009;3(1):45-50.
[301] Shearer GC, Pottala JV, Hansen SN, et al. Effects of prescription niacin and omega-3 fatty acids on lipids and vascular function in metabolic syndrome: a randomized controlled trial. J Lipid Res 2012;53(11):2429-35.
[302] Lamon-Fava S, Diffenderfer MR, Barrett PH, et al. Extended-release niacin alters the metabolism of plasma apolipoprotein (Apo) A-I and ApoB-containing lipoproteins. Arterioscler Thromb Vasc Biol 2008;28(9):1672-8.
[303] Jin FY, Kamanna VS, Kashyap ML. Niacin decreases removal of high-density lipoprotein apolipoprotein A-I but not cholesterol ester by Hep G2 cells. Implication for reverse cholesterol transport. Arterioscler Thromb Vasc Biol 1997;17(10):2020-8.
[304] Ganji SH, Qin S, Zhang L, et al. Niacin inhibits vascular oxidative stress, redox-sensitive genes, and monocyte adhesion to human aortic endothelial cells. Atherosclerosis 2009;202(1):68-75.
[305] Millar JS, Packard CJ. Heterogeneity of apolipoprotein B-100-containing lipoproteins: what we have learnt from kinetic studies. Curr Opin Lipidol 1998;9(3):197-202.

[306] Wood RJ, Volek JS, Liu Y, et al. Carbohydrate restriction alters lipoprotein metabolism by modifying VLDL, LDL, and HDL subfraction distribution and size in overweight men. J Nutr 2006;136(2):384-9.
[307] Tall AR. Exercise to reduce cardiovascular risk--how much is enough? N Engl J Med 2002;347(19):1522-4.
[308] Hernandez M, Wright SD, Cai TQ. Critical role of cholesterol ester transfer protein in nicotinic acid-mediated HDL elevation in mice. Biochem Biophys Res Commun 2007;355(4):1075-80.
[309] Watts GF, Chan DC. Of mice and men: blowing away the cobwebs from the mechanism of action of niacin on HDL metabolism. Arterioscler Thromb Vasc Biol 2008;28(11):1892-5.