The Effect of Exercise on Paraoxonase-1 Activity and Lipid Profile in Obesity and Insulin Resistance Conditions

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Introduction

A sedentary lifestyle is associated with an increased risk of cardiovascular diseases and metabolic syndrome. On the other hand, regular physical activity is a protective factor against the occurrence and progression of these diseases and is associated with decreased blood pressure, maintenance of an appropriate body composition, lipid profile improvement, regular exercise improves the lipid profile and increases the paraoxonase-1 (PON-1) activity. PON-1 interacts with high-density lipoprotein (HDL) and, in the presence of calcium, hydrolyzes free radicals, prevents low-density lipoprotein (LDL) oxidation, maintains homocysteine structure in the blood, and inhibits hemoglobin glycation. These factors explain one of the beneficial effects of regular exercise on prevention of cardiovascular diseases. In addition, there is a positive relationship between decreased PON-1 activity and the occurrence of cardiovascular diseases, renal failure, gastric cancer, dyslipidemia, insulin resistance, and even Alzheimer’s disease. Therefore, this study was conducted to evaluate the effect of physical activity on the PON-1 activity and lipid profile. Regular physical activity increased HDL and PON-1 activity in patients with metabolic syndrome. Since PON-1 binds to HDL and increased HDL probably increases the PON-1 activity as well. This finding suggests that regular exercise decreases the effect of one bout exercise on PON-1 response. In addition, in order to improve metabolic syndromes, it is advised to perform aerobic exercise for 150 minutes per week with an intensity of 40-60% of the heart rate reserve (HRR). The exercises should be preferably performed in 3-5 sessions per week according to the intensity. Based on the disease progression, type of consumed drugs, and certain considerations in each group of patients, aerobic, resistance, and flexibility exercises can be performed by using large muscle groups in a continuous training mode. However, in dyslipidemia, continuous aerobic exercises are preferred.

Keywords: Physical activity, Diabetes, Paraoxonase, Lipid profile

Abstract

In the absence of insulin, regular physical activity facilitates the glucose entry into the cell via affecting several signaling pathways. Moreover, regular exercise improves the lipid profile and increases the paraoxonase-1 (PON-1) activity. PON-1 interacts with high-density lipoprotein (HDL) and, in the presence of calcium, hydrolyzes free radicals, prevents low-density lipoprotein (LDL) oxidation, maintains homocysteine structure in the blood, and inhibits hemoglobin glycation. These factors explain one of the beneficial effects of regular exercise on prevention of cardiovascular diseases. In addition, there is a positive relationship between decreased PON-1 activity and the occurrence of cardiovascular diseases, renal failure, gastric cancer, dyslipidemia, insulin resistance, and even Alzheimer’s disease. Therefore, this study was conducted to evaluate the effect of physical activity on the PON-1 activity and lipid profile. Regular physical activity increased HDL and PON-1 activity in patients with metabolic syndrome. Since PON-1 binds to HDL and increased HDL probably increases the PON-1 activity as well. This finding suggests that regular exercise decreases the effect of one bout exercise on PON-1 response. In addition, in order to improve metabolic syndromes, it is advised to perform aerobic exercise for 150 minutes per week with an intensity of 40-60% of the heart rate reserve (HRR). The exercises should be preferably performed in 3-5 sessions per week according to the intensity. Based on the disease progression, type of consumed drugs, and certain considerations in each group of patients, aerobic, resistance, and flexibility exercises can be performed by using large muscle groups in a continuous training mode. However, in dyslipidemia, continuous aerobic exercises are preferred.

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and decreased type 2 diabetes mellitus signs (1). Moreover, regular physical exercise has positive effects on the metabolism of lipoproteins including decreasing plasma triglyceride (TG) and increasing the HDL-C concentration. Elevated HDL levels increase its antioxidant properties and protective effects against cardiovascular disease (2). HDL has anti-atherogenic and anti-LDL oxidation properties (3). The HDL antioxidant properties are related to the paraoxonase activity (aryl dialkyl phosphate) which can inhibit a number of phospholipids biological oxidizers (4). In addition, paraoxonase as an antioxidant and other antioxidants maintain the structure of homocysteine and prevent from hemoglobin destruction (5). Therefore, from a physiologic perspective and for research purposes it seems that measurement of the PON activity along with lipid profile is important.

**Function and Biochemical Structure of PON enzyme**

The current knowledge of PON indicates its anti-atherogenic properties as far as lack of PON gene expression is known risk factor of cardiovascular disease. The PON enzyme belongs to the family of calcium-dependent enzymes, including PON-1, PON-2, and PON-3 (6). Different PON enzymes are categorized according to their tissue distribution and expression, resulting in their physiologic differences. Most studies on PON-2 and PON-3 have mentioned their cell protective role as well as their role in cellular organelles like the mitochondrion (7) although the activity of one type of PON may be more prominent in different human or animal species. The PON-1 enzyme binds to HDL and has apo-protein AI and J (8). This enzyme is responsible for the antioxidant properties of HDL and PON-1 has a protective role against the progression of atherosclerosis (9,10). This finding was confirmed in rats which the expression of this gene was suppressed (4) or augmented (11). As that mentioned to the prevention of lipid oxidation, since oxidative stress is associated with progression of atherosclerosis, it seems that a balance between the sources of free radicals production and antioxidant activity, including increased PON-1 activity, plays a key role in decreasing cardiovascular diseases. Although PON is an important antioxidant, its measurement alone cannot show the antioxidant capacity of the body and other enzymatic, nutritional, and non-enzymatic antioxidants should also be evaluated.

The PON is a glycoprotein with 354 amino acids and a molecular weight of 44 kDa that can reversibly bind to organophosphorus compounds and hydrolyze them in the presence of 2 calcium ions (12). Since PON was first discovered during the breakdown of a pesticide known as paraoxon and its activity was studied in patients undergoing dialysis (13). The PON-1 is synthesized in the liver and binds to HDL unsaturated phospholipid chains (14). Changes in the size and volume of HDL markedly affect the antioxidant capacity of the PON-1, leading to controversies in the results of different studies (15). PON-1 also has catalytic properties like paraoxonase, arylesterase, diazoxonase, and lactonase activity (16). The PON-1 lactonase activity plays an important role in the maintenance of homocysteine (5,17). Simultaneous decreased activity of PON-1 and HDL and increased oxidized LDL are associated with many diseases (15,18). Apo protein-AI and J bind and execute paraoxonase (16,19). It should be noted that PON-1 has different polymorphisms, which its genetic transcription can be affected by age, sex, physical activity, nutrition, and obesity and expressed on chromosome seven at different loci (19-21). A genetic change at position 191 results in the substitution of arginine (B) with glutamine (A), and substitution of methionine with leucine occurs at position 54 (22).

**The Paraoxonase-1 activity and Diseases**

Low PON-1 activity was reported in many diseases like obesity, diabetes, renal insufficiency, cancer, and metabolic syndrome (18,23). Its decreased activity was also showed...
in cerebral vascular diseases, resulting in decreased blood flow to the brain and irreversible changes of the brain tissue, which is an important case of ischemic stroke (24). The PON-1 activity is markedly decreased in diabetic postmenopausal women (25,26) and hyperlipidemia (2). However, some reports showed the activity of the enzyme is more important than the activity of its polymorphism especially in diabetes (27-29). The LDL is oxidized upon entering the cells via lipoxygenase and myeloperoxidase pathways (30). The formation of this pro-inflammatory compound stimulates the immune system and macrophages start digesting this compound, resulting in the production of foam cells and atheroma formation (30). This is the reason why PON-1 is helpful in treatment with statins (31). In some cases, the activity of PON-1 is independent of HDL and the enzymatic activity is low despite adequate HDL concentrations, which is probably more associated with the role of Apoproteins (29). Atherosclerosis is more frequent in diabetic patients (32) probably due to the disturbed lipid profile and some other factors; paraoxonase-1 binds to Apo-AI and prevents LDL oxidation (33,34). In addition coronary artery disease (CAD) is the most common cause of death in diabetic patients (32). In this regard, some studies showed decreased salivary PON-1 activity in type 2 diabetic rats (35) although some other studies showed no differences between patients and controls (28). It is interesting that PON-1 activity decreases even in temporary conditions like pregnancy (36). However, lifestyle modification, even without changing the lipid profile and PON-1 concentration, increases their anti-inflammatory properties (37). Thyroid hormones disorders may decrease PON-1 activity on the metabolism of lipoproteins and mitochondrial activity (38). The oxidant condition and oxidative stress indexes were significantly worse in children with metabolic syndrome in comparison with control children, which could be the risk factor of many diseases in the future of their lives (39). In addition, there is a significant association between decreased expression of PON-1 gene and ovarian disorders in women (40). It is interesting that many studies have introduced hypertension, type 2 diabetes, obesity, dyslipidemia, decreased HDL, and oxidative stress as the risk factors of Alzheimer’s disease (41). It also was reported that increased HDL and Apo-AI decrease the risk of Alzheimer’s disease (42).

Lipid profile, PON-1 activity and herbal medicine
Many studies were done on the effects of medicinal plants, especially with exercise on diabetes and obesity (43,44). The increased activity of PON-1 following the consumption of herbal medicine is associated with vitamin E in their oil. Vitamin C, E, and polyphenols affect the activity of PON-1 (45,46). It is possible that some herbal medicines like cuminum cyminum L. affect blood glucose via increasing the insulin level (47). The foods containing flavonoids like pomegranate juice (48) and green tea (49) can affect PON-1 due to the number and position of their hydroxyl groups. It was previously reported that garlic (50) and hazelnut (51) decrease LDL and total cholesterol. Some herbal medicine like curcumin can regulate blood sugar, prevent liver damage and inflammation due to their antioxidant properties including PON-1 activity (52-55). It was recently reported that one of the most important activities of curcumin is preventing activation of NFκβ inflammatory pathways and increasing antioxidant defense (56,57). In this regard, there are confirmed reports on the antioxidant (58), anti-inflammatory and anti-cancer (59), hepatic protective (60), blood glucose lowering (53) and lipid lowering (54) effects of curcumin. The most interesting finding regarding turmeric and its effects on PON-1 and metabolic syndrome is prevention of hemoglobin glycation because free radicals disturb the cytochrome structure of heme-containing cells like hemoglobin compounds.

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Turmeric prevents this injury due to its antioxidant properties and its effect on increasing PON-1 and therefore plays a role in controlling hemoglobin glycation. On the other hand, high levels of cholesterol change the hemoglobin structure. Therefore, increased antioxidant defense, especially PON-1, prevents both hemoglobin glycation and lipid oxidation. In addition, due to lactonase effects, PON-1 maintains the homocysteine structure and breakdown of homocysteine-thiolactone structure. As a result, PON-1 is an important cardiovascular protective factor and plays a significant role in controlling metabolic syndromes (5,17).

Effect of exercise on Lipid profile and PON-1 activity

Because extreme exercise in terms of oxidative stress (62) various aspects the effects of exercise on PON-1 activity were investigated. Theoretically, a single session of intense exercise results in the release of free radicals and increase oxidative pressure (63) while regular exercise acts as a protective and antioxidant factor. As will be mentioned regular exercise is also known as a protective factor against the prevalence and progression of heart diseases. Free radicals increase during a single session of intense exercise and improve antioxidant mechanisms due to adaptation through signaling pathways. It should be noted that among factors affecting the lifestyle, physical activity is one of the regulators of the PON-1 activity. Previous findings have also shown that exercise affects PON-1 activity (62,64,65). However, there is no integrated information in this regard. The effect of physical activity on PON-1 activity may depend on the type and intensity of the exercise (62,64,65). The role on PON-1 exercise-related antioxidant mechanisms is not yet clear and therefore understanding the relationship between physical activity and PON-1 activity may have practical results.

Thomas et al (2002) reported that regular exercise had no effect on PON-1 activity while it increased following one session of intense aerobic exercise and returned to initial levels after 24 hours (66). Some other studies have reported similar effects in rats (67). These findings support the hypothesis that the oxidative stress due to intense physical activity increases lipid peroxidation, resulting in PON-1 activity and oxidant defense effort to prevention of lipid oxidation (68). Notice that separate bouts of intense exercise provide a great source of free radicals, oxidative stress, and lipid peroxidation condition (66). However, adaptation to intense exercise causes in response to single session of intense exercise increases the production of antioxidant agents and decreases lipid peroxidation (69), probably indicating PON-1 activation. It is interesting that this finding is not observed in untrained groups (70). Possibly, after intense exercise lipid oxidation inactivates PON-1 through binding to free active sites and LDL oxidized lipids (71). These findings support the hypothesis that the oxidative stress resulting from physical activity in untrained people increases lipid peroxidation, which is in turn, associated with decreases PON-1 activity (68,72). In fact, oxidative stress somehow engages PON-1 in fighting free radical and lipid peroxidation. In confirmation of these findings, a significant increase in baseline activity of PON-1 is reported in soccer players in comparison with inactive groups (73). This finding suggests that regular exercise decreases the effect of one bout exercise on PON-1 response. Nevertheless, the mechanism of the effect of regular exercise on the changes of PON-1 activity in response to one bout intense exercise is unclear yet.

Moreover, regular exercise improves the antioxidant system (70,72,74), and decreases lipid peroxidation (72). However, the effects of regular exercise, even in trained people, are not fully protective against the effects of oxidative stress resulting from one bout intense exercise (74). The activity of antioxidants increases following regular exercise but increased oxidative stress resulting from intense physical activity is
probably more rapidly restored to baseline levels in trained people (74). Each session of physical activity results in the production of free radicals that trigger the production of antioxidants (74) (PON-1 for example). The factors that play a major role in the synthesis of PON-1 are affected by IL-6 and other inflammatory pathways, which is an acceptable explanation for decreased PON-1 activity and Intense physical activity increases oxidized phospholipids, which results in the secretion of pro-inflammatory cytokines like IL-6 and a decrease in PON-1 activity in rats (37,75). Little information is available on the effect of biological rhythms on PON-1 activity; however, the interaction is possible considering the effect of some hormones like estrogen on PON activity since estrogen decreases free radicals. It may be one of the reasons for the lower prevalence of cardiovascular diseases in premenopausal women versus men (25).

Roberts et al (2006) evaluated the effect of diet control and physical exercise on inflammatory and anti-inflammatory properties of HDL in men with cardiovascular risk factors (37). After three weeks, the anti-inflammatory effect of HDL-C was increased despite its low concentration. These findings suggest that rapid lifestyle changes improvement the effectiveness of HDL despite its low amount and changes its activity from pre-inflammatory to anti-inflammatory (37). It was reported that combination of aerobic exercise with 65% of VO$_2$max and niacin therapy increases the concentration and activity of PON-1 in metabolic syndrome patients (76). Moreover, it has been shown that aerobic exercise increases PON-1 in obese and people with metabolic syndrome (77,78). These findings have also been observed in cardiovascular rehabilitation groups (79). However, a study showed no marked changes in HDL and PON-1 levels even 6 months after aerobic exercise in obese women despite a considerable weight loss although the functional indexes of HDL and PON-1 improved (80). This finding suggests that a decrease in the HDL-C level is probably not a disease risk factor in obese diabetic or non-diabetic women if the lifestyle and diet are modified.

Nouno et al (2012) evaluated the relationship of PON-1 activity and lipid profile with cardiac diseases and the effect of flaxseed supplements on controlling dyslipidemia (81). Flaxseed has received a lot of attention in cardiac diseases because it is an important source of alpha-linolenic acid and fiber. Flaxseed supplementation in combination with regular exercise increased HDL and PON-1 significantly and decreased other cardiac markers like IL-1$\beta$ and TNF- $\alpha$. Through flaxseed supplementation, regular physical activity improves the lipid profile and decreases the risk of cardiovascular diseases. The authors concluded that increased HDL and PON-1 resulting from the mutual effect of flaxseed supplement and physical exercise was protective against detrimental effects of acute myocardial ischemia (81).

Briefly, numerous studies have shown increased PON-1 activity following regular physical exercise. The most important point regarding the relationship between regular exercise and increased PON-1 activity is long-term adaptation and increased HDL in response to regular exercises. Since PON-1 binds to HDL and increased HDL probably increases the PON-1 activity, as well. However, no integrated information is available on changes in PON-1 activity in other types of exercise like a single session of intense exercise. Although adaptation with physical activity due to the impact of changes in lifestyle, environmental and genetic factors such as polymorphisms of PON-1 (65), But it has been shown that people who have had regularly exercise, have a less oxidative stress and more PON-1 activity in response to one bout intense exercise (64). Perhaps some of the adaptations with training that reduce the need to insulin and improve physical fitness in patients with type-1 diabetes (82). It has also been reported that improve the serum lipid profile in response to concurrent exercise.
Physical activity and Signaling Pathways affecting glucose uptake

The pathogenesis of metabolic syndromes is lack of blood glucose regulation and increased oxidative stress in these patients. Since the liver has a prominent role in the production of blood glucose, increased blood glucose disturbs oxidation-reduction (redox) reactions in hepatocytes, leading to the production of free radicals. Therefore, blood glucose control alone does not prevent secondary complications. Even blood glucose regulating drugs have side effects and have no role in preventing secondary complications. However, attention has been paid to physical activity due to its insulin-like effects. Insulin is a known factor in glucose uptake by cells but its secretion stops during exercise. On the other hand, insulin is necessary for glucose to enter muscle cells. Interestingly, several folds increase glucose uptake during exercise. Therefore, it can be concluded that factors other than insulin are involved in glucose entry into the cells. Care must be taken that physical activity and insulin have synergistic effects and markedly decrease blood glucose. It has been well demonstrated that muscular contraction has insulin-like effects through known and unknown pathways. However, up-regulation of insulin receptors occurs after and not during physical activity, which is desirable and remains stable in the body for hours after the exercise, and lowers the need for drugs (85,86). On the other hand, some studies have shown that increase TNF-α following muscular injury in intense exercises containing eccentric contraction results in the blockade of signaling pathways (86) and may be a reason for fatigue. Apart from known hormonal pathways that actually augment or inhibit cellular and molecular pathways, the most important signaling pathway of insulin stimulation is formed in response to increased metabolic needs that activates a complex of proteins, resulting in GLUT-4 translocation and even an increase in its gene expression even after the exercise (87). One of the most important advantages of exercise in diabetic patients is increasing the number and sensitivity of insulin receptors after physical activity. Following a training session, the glycogen in the muscle is depleted, resulting in an increase in glycogenesis enzymes. In fact, glycogen depletion is the main factor for glucose entry into the cell because if all conditions are met but the cell does not need glucose, it will not enter the cell (43,87).

Evaluations have shown that increased calcium ion during muscular contractions in physical activity play an important role in glucose entry during exercise. Calcium plays its role though activation of protein kinase C (PKC) and Calcium-calmodulin (CaMK) second messenger mechanism (88). Another mechanism is the activity of phosphatidylinositol kinase-3 (PI3K) enzyme that is more controlled by insulin than physical activity. The absence of insulin or any failure in its receptors results in the inhibition of this enzyme and inhibition of glucose entry into the cell. In the breakdown of membrane phospholipids as a second messenger mechanism, PIP2 breaks down to IP3 and DAG. The IP3 pathway results in calcium release with known mechanisms while the DAG pathway results in the production of PKC and prostaglandins, especially E2 (89). Some studies have shown that inhibition of PKC enzyme disturbs the process of glucose transpiration. This mechanism is one of the
mechanisms of the effect of contraction and more contraction enhances glucose entry. In addition, the NO activity is increased during exercise. It has been shown that inhibition of NO production and adenosine disturbs glucose entry (90) which is probably due to affecting the blood flow to the muscle. On the other hand, NO activates the signaling pathways of glucose entry into the cell, including (mitogen activated protein kinase) MAPK, JNK, ERK, and P38 (91,92). In addition to the above pathways, it has been mentioned that during exercise, due to ATP consumption and decreased ATP to ADP ratio, adenosine monophosphate-activated protein kinase (AMPK), which works under the influence of the ATP to ADP ratio, enhances glucose uptake (93).

Conclusion and exercise prescription
Although many studies have evaluated the effect of physical exercise on PON-1 activity in patients and healthy individuals, it is hard to reach an integrated conclusion because PON-1 activity is regulated by many factors and exercise is one of them. The possible reasons were discussed in the previous section. Attention should be paid to the FITT-VP components of exercise prescription including Frequency, Intensity, Time (duration), Type (mode), Volume (quantity), and Progression proposed by American College of Sports Medicine (ACSM) (94-96). Therefore, it should be noted that before exercise prescription, the person should be evaluated for physical capabilities and biochemical indexes. There is need for exercise testing beyond the PAR-Q questionnaire, medical history, and biochemical tests before exercises. However, people with cardiovascular diseases should undergo graded exercise tests and electrocardiography before exercise although they do not eliminate the risk of accidents (94-96).

According to the ACSM’s guidelines, aerobic, resistance, and flexibility exercises can be performed in diabetic patients with a frequency of 3-5 sessions per week and intensity of 40-60% of HRR or a score of 11-13 in the Borg Rating of Perceived Exertion Scale (6 to 20 points). These activities should be performed for 150 minutes per week in separate sessions and should preferably increase to 300 minutes. These activities should be performed according to the person’s preference using large muscle groups in a rhythmic and continuous mode. Some studies have shown that a combination of aerobic and resistance training has better results (82). The person should not be inactive for two consecutive days. Moreover, attention should be paid to the goal of exercise in these people, i.e. cardiovascular preparedness, weight loss, etc. blood glucose should be monitored regularly to prevent hypoglycemia. These patients should use special ointments and socks for a better hand and foot care (82). It should be borne in mind that glucose metabolism defects may result in hyperglycemia with ketosis (97). Moreover, dehydration following polyuria can be an effective factor in lack of temperature regulation and electrolyte balance. Diabetic patients with retinopathy should do very light exercise to prevent excessive increase in blood pressure, retinal injury, and severe hemorrhage (97). Improved glucose tolerance and increased insulin sensitivity are observed in patients with type II diabetes and the need for insulin decreases in type 1 diabetic patients (82). A diabetic patient with neuropathy or ocular laser surgery should avoid from resistance training (97). Hypoglycemia is the most important complication in these patients which is more often seen in patients receiving insulin or other glucose lowering drugs (94-96).

Abnormal concentrations of lipids and lipoproteins are known as dyslipidemia. Dyslipidemia occur when the LDL concentration increases or the HDL concentration decreases (94-96). In summary, aerobic training is more recommended in lipid disorders (84). The ACSM also recommends aerobic training for patients with lipid disorders; the person should do aerobic
training 5 days a week at 40-70% of HRR. It is recommended to exercise 30-60 minutes per session. These people should do aerobic training 250 minutes per week to both decrease the fat mass and change the lipid profile. If the person has, other disorders like diabetes or hypertension besides lipid disorders, its certain considerations should be regarded. Some lipid lowering agents as statins cause muscle weakness, severe muscle soreness, or myalgia, which require exercise modification.

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