PARAOXONASE1 STATUS IN HEALTHY THAI POPULATION AND EFFECT OF FENOFIBRATE THERAPY ON PARAOXONASE1 STATUS IN PATIENTS WITH LOW HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS

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ABSTRACT

The aims of this study are to determine the *PON1* polymorphisms and to investigate the influence of these polymorphisms on serum PON1 concentration and PON1 activity in healthy Thais. In addition, the effect of fenofibrate on PON1 levels and the influence of these *PON1* polymorphisms on the therapeutic response of fenofibrate in patients with low HDL-C levels was also investigated.

In healthy Thais, genotype and allele frequencies of the *PON1* L55M, Q192R, T-108C and G-909C polymorphisms were 89.55%LL, 10.45%LM (L=0.95, M=0.05), 50.0%QQ, 41.79%QR, 8.21%RR (Q=0.71, R=0.29), 55.22%TT, 41.05%CT, 3.73%CC (T=0.76, C=0.24), 55.22%CC, 34.33%CG, and 10.45%GG (C=0.72, G=0.28), respectively. The *PON1* L55M polymorphism was associated with a significant variation in serum PON1 concentration. The levels of PON1 activity towards paraoxon and diazoxon were significantly influenced by the *PON1* Q192R and G-909C polymorphisms. These findings indicate the physiological relevance of the *PON1* polymorphisms in healthy Thais since they are associated with significant differences in serum PON1 levels.

Fenofibrate significantly decreased triglycerides, non-HDL-C, oxidized LDL and apo B levels whereas HDL-C and apo AI levels were significantly increased. Interestingly, there were significant increases in both PON1 concentration and activity. The therapeutic response of lipid parameters to fenofibrate was independent of *PON1* polymorphisms whereas the *PON1* Q192R and T-108C polymorphisms affected the therapeutic response of PON1 levels to fenofibrate therapy. The 192QQ and –108TT genotypes had higher increase in PON1 levels in response to fenofibrate. Fenofibrate treatment in patients with low HDL-C levels not only reduces atherogenic lipids and increases atheroprotective lipids but also increases PON1 levels. Increasing PON1 levels by fenofibrate may play an important role in decreasing LDL oxidation.

KEY WORDS: PARAOXONASE1/ POLYMORPHISMS/ FENOFIBRATE/ LOW HDL-C/ OXIDIZED LDL

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สถานะภาพของเอนไซม์พาราออกซอนเนส1ในประชากรไทยปกติและผลของการรักษาด้วยยาฟีโนไฟเบรตต่อสถานะภาพของเอนไซม์พาราออกซอนเนส1ในผู้ป่วยที่มีระดับเอชดีแอลโคเลสเตอรอลต่ำ (PARAOXONASE1 STATUS IN HEALTHY THAI POPULATION AND EFFECT OF FENOFIBRATE THERAPY ON PARAOXONASE1 STATUS IN PATIENTS WITH LOW HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS)

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาและทดสอบอิทธิพลของ *PON1* polymorphisms ที่มีต่อ PON1 concentration และ PON1 activity ในคนไทยปกติ นอกจากนี้ยังศึกษาผลของยา fenofibrate ต่อระดับ PON1 และทดสอบอิทธิพลของ *PON1* polymorphisms ต่อการตอบสนองของการรักษาในผู้ป่วยที่มีระดับ HDL-C ต่ำ

ในคนไทยปกติ ความถี่ genotype และ allele ของ *PON1* L55M, Q192R, T-108C และ G-909C polymorphisms คือ 89.55%LL, 10.45%LM (L=0.95, M=0.05), 50.0%QQ, 41.79%QR, 8.21%RR (Q=0.71, R=0.29), 55.22%TT, 41.05%CT, 3.73%CC (T=0.76, C=0.24), 55.22%CC, 34.33%CG, 10.45%GG (C=0.72, G=0.28) ตามลำดับ *PON1* L55M polymorphism มีความสัมพันธ์กับความแตกต่างของ PON1 concentration ในซีรัม ระดับ PON1 activity ต่อ paraoxon และ diazoxon ถูกควบคุมโดย *PON1* Q192R และ G-909C polymorphisms การค้นนี้พบบ่งชี้ถึงบทบาททางสรีรวิทยาของ *PON1* polymorphisms ในคนไทยปกติที่มีความสัมพันธ์กับความแตกต่างของระดับ PON1 อย่างมีนัยสำคัญทางสถิติ

ยา fenofibrate มีผลลดระดับ triglycerides non-HDL-C oxidized LDL และ apo B ขณะที่ระดับ HDL-C และ apo AI มีค่าเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ที่น่าสนใจคือมีการเพิ่มขึ้นของทั้ง PON1 concentration และ activity การตอบสนองของระดับไขมันในการรักษาด้วยยา fenofibrate ไม่ขึ้นกับ *PON1* polymorphisms ขณะที่ *PON1* Q192R และ T-108C polymorphisms มีผลต่อการตอบสนองของระดับ PON1 พบว่าการเพิ่มของระดับ PON1 มากใน 192QQ และ –108TT genotypes การรักษาด้วยยา fenofibrate ในผู้ป่วยที่มีระดับ HDL-C ต่ำ ไม่เพียงลด atherogenic lipids และเพิ่ม atheroprotective lipids แต่ยังมีผลเพิ่มระดับ PON1 โดยยา fenofibrate อาจมีบทบาทสำคัญในการลดการออกซิเดชั่นของ LDL-C

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LIST OF ABBREVIATIONS

α	=	Alpha
ε	=	Molar extinction coefficient
μg	=	Microgram
μL	=	Microliter
μM	=	Micromolar
μmol	=	Micromole
%	=	Percent
ABCA1	=	Adenosine triphosphate binding cassette transporter A1
ALT	=	Alanine aminotransferase
Apo (a)	=	Apolipoprotein (a)
Apo AI	=	Apolipoprotein AI
Apo AII	=	Apolipoprotein AII
Apo B	=	Apolipoprotein B
Apo CIII	=	Apolipoprotein CIII
AST	=	Aspartate aminotransferase
ATP	=	Adult Treatment Panel
BMI	=	Body mass index
BW	=	Body weight
°C	=	Degree celcius
CETP	=	Cholesterylester transfer protein
CHD	=	Coronary heart disease
CK	=	Creatinine kinase
DBP	=	Diastolic blood pressure
dNTP	=	Deoxynucleotide triphosphate
dL	=	Deciliter

LIST OF ABBREVIATIONS (CONTINUED)

DMSO	=	Dimethyl sulfoxide
ELISA	=	Enzyme-linked-immunosorbent assay
FBG	=	Fasting blood glucose
HDL-C	=	High-density lipoprotein cholesterol
HMG-CoA	=	3-Hydroxy-3-methylglutaryl coenzyme A
hr	=	Hour
hs-CRP	=	High sensitivity C reactive protein
IL	=	Interleukin
kDa	=	Kilodalton
kg	=	Kilogram
L	=	Liter
LDL-C	=	Low-density lipoprotein cholesterol
Lp (a)	=	Lipoprotein (a)
LPL	=	Lipoprotein lipase
mg	=	Milligram
min	=	Minute
mL	=	Milliliter
mmHg	=	Millimeter mercury
mM	=	Millimolar
mmol	=	Millimole
nmol	=	Nanomole
NCEP	=	National Cholesterol Education Program
OD	=	Optical density
OxLDL	=	Oxidized low-density lipoprotein
PCR	=	Polymerase chain reaction
PON	=	Paraoxonase
PBS	=	Phosphate buffer saline
PPAR	=	Peroxisome proliferator activated receptor

LIST OF ABBREVIATIONS (CONTINUED)

RCT	=	Reverse cholesterol transport
rpm	=	Revolutions per minute
SD	=	Standard deviation
SR-BI	=	Scavenger receptor BI
SBP	=	Systolic blood pressure
TC	=	Total cholesterol
TG	=	Triglycerides
TMB	=	3,3',5,5'-Tetramethylbenzidine
VLDL	=	Very low-density lipoprotein
vs	=	Versus
ULN	=	Upper limit normal
WHR	=	Waist hip ratio

CHAPTER I
INTRODUCTION

Numerous clinical and epidemiological studies have established that low levels of high-density lipoprotein cholesterol (HDL-C) are strongly and independently associated with an increased risk of coronary heart disease (CHD) (1-5). Those studies revealed that every 1 mg/dL increase in HDL-C was associated with CHD risk reduction of 2% in men and 3% in women after correction for other CHD risk factors. However, about 40% of patients with CHD have low-density lipoprotein cholesterol (LDL-C) levels below 130 mg/dL (3.4 mmol/L), and most of these patients also have low HDL-C levels with or without increased levels of triglyceride (TG) (6, 7). The level of HDL-C is being suggested increasingly as a key variable involved in vascular risk assessment, and raising HDL-C is a concomitant goal of therapy (8-12). The importance of low HDL-C levels (< 40 mg/dL or 1.03 mmol/L) as a risk factor for the development of CHD, is recognized by British, European, and US guidelines (9-11). The recently published National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, ATPIII) guidelines (11) place greater emphasis on low HDL-C levels than the previous version (12) and have reset for a higher cutpoint to define "low HDL-C" from 35 mg/dL (0.9 mmol/L) to 40 mg/dL (1.03 mmol/L). Furthermore, the guidelines recommend the use of drugs for raising HDL-C levels in individuals with isolated low HDL-C levels and CHD or CHD risk equivalents (11).

High-density lipoprotein exerts various potentially antiatherogenic properties. A mechanism for the effect most attention has been focused on the role of HDL in reverse cholesterol transport (RCT) (13, 14). However, the evidences from several studies have suggested a role for HDL in the protection of LDL against oxidative modification (15-19), which is now generally accepted to be central to the initiation

and progression of atherosclerosis (20, 21). Various factors including apolipoprotein AI (apo AI), paraoxonase1 (PON1), platelet activating factor acethyl hydrolase (PAF-AH) and lecethin cholesterol acyltransferase (LCAT) can contribute to antioxidant effect of HDL (22). Numerous studies have indicated that PON1, a HDL-associated enzyme, is largely responsible for HDL's antioxidative property (23-28). The potential of PON1 to protect LDL against oxidative modification was reinforced in animal studies. HDL isolated from PON1 knockout mice was unable to prevent LDL oxidation in a cell co-culture model of the artery wall, and both the HDL and LDL isolated from PON1 knockout mice were more susceptible to oxidation by co-culture cells as compared to control mice. In this model, both PAF-AH and LCAT were unable to prevent LDL oxidation in the absence of PON1 (29, 30).

PON1 is a calcium-dependent HDL-associated ester hydrolase. It is located in a subfraction of HDL that contained apo AI and clusterin (apo J) (31, 32). Paraoxonase is so called because paraoxon is the substrate commonly used to measure enzyme activity. PON1 activity is found in a variety of mammalian tissues, particular serum, liver, kidney, and small intestine (32). The *PON* gene family contains at least three members, including *PON1*, *PON2*, and *PON3*, which are located adjacent to each other on chromosome 7 in humans (33). The physiological roles of the PON2 and PON3 gene products are still unknown. Moreover, PON2 and PON3 activity or mass have not been evaluated due to the lack of functional assays (34).

In 1991, Mackness *et al*. reported that PON1 inhibits the copper-catalyzed oxidation of LDL (23). This provided the first molecular hypothesis for a specific mechanism whereby HDL might affect LDL oxidation. In addition, PON1 can remove oxidized phospholipids from LDL (25). These oxidized lipids activate inflammatory genes and promote the adhesion of monocytes to endothelium, a key event in monocyte recruitment into early atherosclerotic lesion. Thus, PON1 may alter the risk for vascular disease by cleansing LDL of oxidized lipids *in vivo*.

The PON1 activity is genetically determined and is reported to have marked interindividual and racial variation. This variation is due to *PON1* polymorphisms. Two major coding region polymorphisms that have been extensively studied are amino acid substitutions at position 55 [leucine (L) to methionine (M)] and at position 192 [glutamine (Q) to arginine (R)] (33, 35). 192QQ, an isoform with low activity

towards paraoxon, has a glutamine at position 192 whereas the 192RR isoform, with high activity towards paraoxon, contains an arginine at that position (33, 36). *PON1* L55M polymorphism has a lesser effect on PON1 activity. The *PON1* polymorphisms have been suggested to be an independent risk factor for CHD (37-43). Both the 55LL and 192RR genotypes have been shown to be associated with increased susceptibility to CHD. Several studies have shown that serum PON1 activity is reduced in CHD, diabetes mellitus, familial hypercholesterolemia and chronic renal failure, diseases that are associated with accelerated atherosclerosis (43-46). The *PON1* polymorphisms have also been found in regulatory region and have a functional effect on PON1 expression. These polymorphisms are at position –108 [thymine (T) or cytosine (C)], -162 [adenine (A) or guanine (G)], -909 [guanine (G) or cytosine (C)], where the base immediately before the start codon is numbered as "-1" (47).

Previous evidence has demonstrated that atherosclerosis is not simply a disease of lipid deposition. Inflammation plays a role in the initiation, progression, and destabilization of atheromas (48). C-reactive protein (CRP) is a circulating acute-phase reactant that reflects active systemic inflammation. It is synthesized and secreted by hepatocytes (49). Several large-scale prospective epidemiological studies have shown that serum levels of high sensitivity CRP (hs-CRP) are a strong independent predictor of a risk of future myocardial infarction (MI), stroke, peripheral arterial disease, and vascular death among individual without known cardiovascular disease (50-54). In addition, among patients with acute coronary ischemia, stable angina pectoris, and a history of MI, levels of hs-CRP have been associated with increased vascular event rates (55-57).

Thus, the potential pharmacological agents for raising HDL-C and PON1 activity, and reducing oxidized LDL (oxLDL) and hs-CRP levels could be an additional strategy in the prevention of cardiovascular disease.

Numerous trials with the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) and the fibrates demonstrated a significant reduction in cardiovascular events (58-62). Although, the majority of the effects could be ascribed to the beneficial effect on the lipid profiles, these drugs might have additional effects that could confer benefit such as protective action against oxidative modification of serum lipoproteins. However, there were a few studies of the lipid-lowering drugs on

PON1 activity. Recently, simvastatin, gemfibrozil and micronized fenofibrate therapy have been reported to raise PON1 activity in patients with familial hypercholesterolaemia, hyperlipidaemia and coronary heart disease, respectively (63-65). However, this effect was not found in bezafibrate and gemfibrozil therapy in patients with type IIb hyperlipoproteinaemia, and ciprofibrate therapy in patients with familial hyperlipoproteinaemia (66, 67).

Based on the hypothesis that PON1 has a protective function against CHD development, the influence of PON1 activity might be of major importance for the efficacy of its protective function, and since the action of fibrates via peroxisome proliferator-activated receptor-alpha (PPARα) on increasing HDL-C levels, where PON1 is also associated. Microcoated fenofibrate is a new preparation which has improved bioavailability and pharmacokinetics. The first aim of this study was to determine the four reported *PON1* polymorphisms in both coding region (L55M and Q192R) and regulatory region (T-108C and G-909C) and to investigate the association of these polymorphisms with PON1 concentration and activity, and lipid profiles in healthy Thai population. The second aim was to investigate whether fenofibrate therapy is associated with changes in PON1 concentration and activity and to analyze the influence of *PON1* polymorphisms on the response of PON1 levels to fenofibrate therapy in patients with low HDL-C levels.

Objectives

1. To determine PON1 status (activity, concentration and genotypes) and lipid profiles in healthy Thai population and patients with low HDL-C levels.
2. To analyze the association between PON1 status and lipid profiles in healthy Thai population and patients with low HDL-C levels.
3. To determine the effect of fenofibrate on PON1 concentration and activity, lipid profiles, and hs-CRP in patients with low HDL-C levels.
4. To analyze the influence of *PON1* polymorphisms on the response of PON1 concentration and activity to fenofibrate therapy in patients with low HDL-C levels.

CHAPTER II
LITERATURE REVIEW

1. Low high-density lipoprotein cholesterol

Low level of HDL-C is an important risk factor for CHD, which has been found in more than 40% of patients experiencing MI (6, 7). Low HDL-C is the most common lipoprotein abnormality found in men with early atherosclerosis, and the inverse relationship between the level of HDL-C and the occurrence of CHD has been noted in numerous epidemiologic studies (5, 68-70).

1.1 Definition of low HDL-C

NCEP-ATPIII sets HDL-C level of < 40 mg/dL as a categorical risk factor and designates it a factor that modifies the LDL-C goal (11). In ATP II, a low HDL-C was defined as a level < 35 mg/dL (12); the setting of this cutpoint was influenced by the concept that low HDL-C is primarily a direct cause of atherosclerotic disease. More recently, the role of HDL-C as an indicator of other risk correlates has been emphasized (71-73). This shift in perception requires a re-examination of the appropriate cutpoint for low HDL-C. Clearly, low HDL-C levels predict CHD at levels above 35 mg/dL (74); this fact combined with the moderate reductions of HDL-C caused by obesity and physical inactivity led the ATP III panel to recognize a some what higher HDL-C level as a categorical risk factor. The level of < 40 mg/dL was set as a low HDL-C in both men and women (11). Women typically have higher HDL-C levels than men, and a cutpoint of < 40 mg/dL will identify more men than women with low HDL-C, i.e., approximately one-third of men and about one-fifth of women in the general population.

1.2 Causes of low HDL-C

There are several factors that contribute to low HDL-C levels as presented in table 1. In general population, about 50% of the variability of serum HDL-C levels derives from genetic factors (75); the other 50% presumably comes from the acquired factors listed in table 1.

Genetic causes of low HDL-C include Tangier disease, familial hypoalphalipoproteinemia, apo AI deficiency, and LCAT deficiency. Tangier disease is a rare autosomal-recessive disorder characterized by decrease cholesterol efflux from peripheral cells resulting in HDL-C and LDL-C levels that are virtually undetectable (76). Patients develop cholesterol deposits in the spleen, liver, lymph nodes, and Schwann cells, and they can have an increased risk of CHD. The genetic mutation of Tangier disease has been localized to the ATP-binding portion of the cholesterol transport molecule ATP binding cassette transporter A1 (ABCA1) (77, 78). This defect results in abnormal cholesterol trafficking and increased degradation of HDL, both resulting in decreased HDL-C levels.

Familial hypoalphalipoproteinemia is a more common disorder of low HDL-C and is caused by a number of heterogenous enzymatic defects, including disorders of ABCA1. Like in Tangier disease, there is increased catabolism of HDL and its precursors (79). Affected individuals have low HDL-C but no peripheral cholesterol deposits.

Deficiency of apo AI can result from increased catabolism or from genetic mutations in the apo AI molecules (80-82). LCAT deficiency, which impairs peripheral cholesterol uptake by HDL, causes rapid catabolism of apo AI and subsequent cholesterol deposition in the cornea, kidneys, and plasma. Premature CHD is variable in both apo AI and LCAT deficiencies. The fact that impaired cholesterol trafficking could lead to premature atherosclerosis in some patients and not in others could indicate that HDL and its subtypes have multifactorial roles in cardioprotection beyond its function in RCT.

Secondary, noninherited causes of low HDL-C levels are common and include the metabolic syndrome (obesity, hypertension, insulin resistance, hypertriglyceridemia, and low HDL-C) (83), smoking (84), physical inactivity (85), and very low-fat diets (86). Medications, including beta blockers, phenothiazines,

androgenic steroids, and progestins, can also contribute to lowering HDL-C levels (87, 88). Moreover, when a person has a genetic predisposition to reduce HDL-C, acquired factors often drive HDL-C to categorically low levels.

Table 1. Causes of low HDL-C levels (73)

Condition associated with decreased HDL-C levels
1. Genetic factors
Tangier disease
Familial hypoalphalipoproteinemia
Apo AI deficiency
LCAT deficiency
2. Environmental factors
Cigarette smoking
Physical inactivity
Very low-fat diet
Very high carbohydrate intake (> 60% of total energy)
3. Diseases
Metabolic syndrome
Type 2 diabetes mellitus
Hypertriglyceridemia
Obesity
4. Drugs
Beta-blockers
Androgens
Progestins
Benzodiazepines

1.3 Low HDL-C as an independent risk factor for CHD

Strong epidemiological evidence links low HDL-C levels to increased CHD morbidity and mortality (3, 5, 69, 70). High HDL-C levels conversely convey reduced risk. Epidemiological data taken as a whole signify that a 1% decrease in HDL-C is associated with a 2-3% increase in CHD risk (1-5). These studies consistently show low HDL-C to be an independent risk factor for CHD. Its independent relationship holds after correction for other risk variables in multivariate analysis. In a subsection of the Framingham Heart Study (FHS), low HDL-C levels, along with high total cholesterol (TC) to HDL-C ratios, were the only independent and significant predictors of CHD (70). In 1988, Gordon *et al.* reported an analysis of several population-based studies, including the FHS, the Lipid Research Clinic Prevalence Mortality Follow-Up Study (LRCF), the Lipid Research Clinics Coronary Primary Prevention Trial (CPPT), and the Multiple Risk Factor Intervention Trial (MRFIT) (69). Each of these studies examined, among other factors, the relationship between plasma lipid levels and the development of CHD. HDL-C levels within the studies were inversely correlated with CHD events, and HDL-C level of < 40 mg/dL was directly related to an increase in CHD. The authors estimated that every 1-mg/dL increase in HDL-C was associated with a CHD risk reduction of 1.9% to 2.3% in men and 3.2% in women. Another study by Goldbourt *et al.* examined whether isolated low HDL-C levels predict CHD events (5). Cardiovascular mortality was 36% higher in men with low HDL-C and normal TC compared with age-matched control subjects with normal HDL-C. This effect was amplified in subjects with a combination of low HDL-C and diabetes mellitus, producing a 65% increase in CHD mortality. These observations support current practice HDL-C screening for risk factor stratification and modification.

1.4 HDL-C raising therapy

1.4.1 Nonpharmacologic therapy

Several nonpharmacologic interventions have been shown to alter HDL-C levels. Diets including judicious amounts of alcohol and those that are high in omega-3 fatty acids have shown benefit in raising HDL-C (89, 90).

Exercise alone has been shown to lead to higher HDL-C levels. In a study of military recruits undergoing abrupt onset of intense physical activity, HDL-C increased by 12 mg/dL over a 12-week period (91). This improvement in HDL-C was independent of associated weight loss or alteration in diet. Furthermore, marathon runners have higher HDL-C levels compared with nonrunners or moderate runners (92). This effect appears to be related to the distance run and hence, duration of exercise. Although there are data indicating that the effect of strenuous exercise on HDL-C is unrelated to any concomitant weight loss, weight loss itself also correlated with improved HDL-C levels, particularly in patients with metabolic syndrome (93).

Smoking cessation is one of the most important life style modifications that can raise HDL-C levels (94). Active cigarette smoking results in approximately 5-mg/dL lower HDL-C levels than that of nonsmoker. Interestingly, cigar and pipe smokers had similar HDL-C levels to nonsmokers in the Framingham Offspring Study, indicating that inhalation of cigarette smoke could be the causative factor in altering lipoprotein concentrations. Smoking cessation ameliorates the risk of decrease HDL-C, with increases in HDL-C levels detected in as little as 2 weeks (95, 96).

1.4.2 Pharmacologic therapy

There are several classes of drugs available for raising HDL-C levels (table 2). These classes include niacin, fibrates, statins, and bile acid sequestrants.

Niacin

Niacin is the most efficacious drug for raising HDL-C levels (97). Niacin decreases the hepatic uptake of HDL precursors, thereby causing more HDL-C to be manufactured and available in the periphery (98). Niacin therapy results in a 20% to 35% increase in HDL-C, is effective in patients with normal or high TG levels, and could be accompanied by a 50% decrease in TG levels (99). *In vivo* kinetic studies attributed the raise of HDL-C by niacin to a decreased fractional catabolic rate of apo AI. Such a mechanism would improve RCT possibly through SR-BI-mediated cholesterol uptake (100). The side effect profile of niacin, including flushing and pruritus, makes this medication somewhat difficult to tolerate in selected patients. These symptoms are often alleviated by premedication with aspirin. Niacin can also

cause gastrointestinal side effects, increased hepatic transaminases, and hyperuricemia (98).

Fibrates

Fibrates regulate HDL metabolism as ligands and activators of the nuclear transcription factor PPAR-α (100-103). This factor binds to PPAR-responsive elements which are found in several pivotal genes of HDL metabolism. Binding of PPAR-α increases the transcription of the genes for apo AI, apo AII, lipoprotein lipase (LPL) and scavenger receptor BI (SR-BI) and ABCA1 and thereby increases HDL production, maturation and catabolism of HDL. Enhanced hepatic secretion of apo AI and apo AII increase HDL production, whereas up-regulation of ABCA1 helps to supply HDL precursor phospholipids and cholesterol from peripheral cells. Furthermore, LPL is upregulated and apo CIII, an inhibitor of LPL, is suppressed by PPAR-α activation. Hence, fibrate treatment enhances lipolysis of TG-rich lipoproteins and, thereby, increases the production of surface remnants and finally the formation of HDL. Stimulation of SR-BI expression will also increase HDL catabolism. The combination of these processes is thought to increase HDL-C levels and to enhance RCT. The increase in HDL-C by fenofibrate has been shown to be related to baseline HDL-C. Individual with low baseline levels of HDL-C experience the strongest increase in HDL-C (104). A small percentage of patients taking fibrates experience gastrointestinal side effects and elevated transaminases, and these medications could potentiate the effect of anticoagulation (104).

Statins

Statins prevent the biosynthesis of hepatic cholesterol via inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and up-regulate the expression of hepatic LDL receptors, thereby leading to reduction in LDL particles (98). Statins up-regulate the synthesis of HDL and its precursors and result in an approximate 5%-10% increase in HDL-C levels (98). There is evidence from *in vitro* experiments that some statins influence both the production and catabolism of HDL. The hypercatabolic effect of statins on HDL metabolism appears to be mediated by up-regulation of SR-BI and down-regulation of ABCA1 (105, 106). Statins-mediated

down-regulation of cholesterol synthesis may enhance selective uptake of HDL-C in the liver through up-regulation of SR-BI, which is expected to increase HDL catabolism and lower plasma HDL-C. Furthermore down-regulation of ABCA1 by inhibition of cholesterol synthesis would reduce HDL maturation and as a secondary effect enhance the catabolism of apo AI. SR-BI up-regulation and ABCA1 down-regulation are expected to lower HDL-C in plasma and cannot explain the statins induced elevation of HDL-C. However, statin-treated patients also had reduced cholesteryl ester transfer protein (CETP) activity, which impairs cholesterol-ester transfer from HDL to apo B containing lipoproteins and, thereby, increases HDL-C (107). Taken together, the *in vitro* data suggest that statins increase HDL-C via enhanced apo AI production that appears to override the HDL-C lowering effect of increased HDL catabolism. Interestingly, there is neither a dose-response relationship nor any positive correlation with the LDL-C lowering effect of statins. It rather appears that those statins which are less efficient in reducing LDL-C are more efficient in increasing HDL-C. In line with this, atorvastatin increases HDL-C at low dosages but decreases HDL-C at high dosages (108). Thus, statins appear to have complex interactions with HDL metabolism. Additionally, because of the divergent effects of statins on the expression of ABCA1 and SR-BI, and because of inhibition of CETP, the effects of statins on RCT from peripheral cells to the liver are difficult to predict (108). Side effects of statins include gastrointestinal upset, myositis, and increased liver transaminases (98).

Bile acid sequestrants

Bile acid sequestrants increase HDL-C levels and can be combined with other medications in the treatment of severe dyslipidemia (98), but their use is limited as a result of significant gastrointestinal side effects and a potential for elevation TG levels.

Table 2. HDL-C raising pharmacotherapy (73)

Drug	Dosage	HDL-C induction
Niacin		
Immediate release	100 mg- 6 g per day	20-35%
Intermediate release	500 mg- 2 g per day	
Sustained release	250 mg- 6 g per day	
Fibrates		
Gemfibrozil	600 mg twice per day	11-25%
Clofibrate	1 g twice per day	
Fenofibrate	100 mg 3 times per day	
Statins		
Atorvastatin	10-80 mg per day	0-9%
Simvastatin	5-80 mg per day	3-16%
Pravastatin	10-80 mg per day	2-12%
Fluvastatin	20-80 mg per day	0-6%
Lovastatin	10-80 mg per day	2-8%
Bile acid sequestrants		
Cholestyramine	4 g-24 g per day	5%
Colestipol	2 g-20 g per day	

1.5 Intervention studies in patients with low HDL-C

Several clinical trials suggest that raising HDL-C levels contributes to decreased risk for CHD (8, 61, 62, 109-111). Nonetheless, in these trials, changes in other lipoproteins also have occurred. Numerous clinical trials have recruited persons with low HDL-C levels and no significant elevations of TG. These trials thus provide information on the benefit of lipoprotein modification in persons with low HDL-C levels.

The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) was a primary prevention study with clinical end points that investigated the effect of lovastatin in patients with low HDL-C and average LDL-C levels (8). In the group receiving lovastatin, all lipid values were improved, including a 6% increase in HDL-C levels. Myocardial infarction or sudden death was decreased by 37% in the lovastatin group. Treatment with lovastatin achieved the greatest risk reduction in cardiovascular event rates in those subjects in the lowest baseline tertile of HDL-C (< 34 mg/dL).

In the Lipoprotein and Coronary Atherosclerosis Study (LCAS), fluvastatin significantly reduced angiographic progression of atherosclerosis among patients with low HDL-C (109). The greatest effect of fluvastatin on vessel diameter was noted in those patients with low levels of HDL-C (< 35 mg/dL) compared with those with high levels of HDL-C (> 35 mg/dL). Additionally, the probability of event-free survival was increased to a greater extent in the group with low HDL-C compared with the group with high HDL-C.

Clinical improvement in subjects with established CHD and lower HDL-C levels has been reported in each of the major statin trials. In the Scandinavian Survival Study (4S) (112), the Long-Term Prevention with Pravastatin in Ischemic Disease (LIPID) trial (113), the Cholesterol and Recurrent Events (CARE) study (114), the West of Scotland Coronary Prevention Study (WOSCOPS) (115) and AFCAPS/TexCAPS (8), statin therapy reduced the risk for coronary events in patients with lower HDL-C levels to approximately that in placebo-treated patients with higher HDL-C concentrations.

The Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) (62) compared treatment with gemfibrozil versus placebo in men with

CHD, LDL-C level < 140 mg/dL and HDL-C level < 40 mg/dL. Changes in lipid profiles after 5 years in those receiving gemfibrozil included a 6% increase in HDL-C level. There was no significant change in LDL-C level. The group receiving gemfibrozil showed a 22% reduction in MI or cardiovascular death, and the risk reduction was greater for the subgroup with lower levels of HDL-C (116).

Likewise, the decrease in major coronary events among asymptomatic men with primary dyslipidemia during gemfibrozil therapy in the Helsinki Heart Study was estimated to be due to partly to an increase in HDL-C levels (110).

A reduction in coronary events in a subgroup of patients with CHD and low HDL-C was also found in the Bezafibrate Infarction Prevention (BIP) Study (61). The study population was similar to that in the VA-HIT trial (62, 111), and after 6 years, the group receiving bezafibrate showed a decrease in LDL-C, TC, and TG, with an increase in HDL-C levels of 18%. There was a nonsignificant (9.4%) reduction in MI or sudden death between the study and control groups. However, the reduction in these primary endpoints was significant in the subgroup receiving bezafibrate who had higher baseline TG and lower HDL-C levels.

Finally, the recent reported High Density Lipoprotein Atherosclerosis Intervention Trial (HAT) examined the effect of the combination of niacin and simvastatin, with or without antioxidant vitamins, in patients with established CHD, low levels of HDL-C (≤ 35 mg/dL in men, ≤ 40 mg/dL in women), and normal LDL-C and TG levels (111). After 38 months, the simvastatin plus niacin group showed a 26% increase in HDL-C, a 42% decrease in LDL-C, and a 36% reduction in TG levels. The antioxidant and placebo group had no significant change in lipoprotein levels. Interestingly, the statin and niacin induced increase in HDL-C was blunted significantly by the addition of vitamins, although there was a similar reduction in LDL-C and TG levels.

2. Paraoxonase

2.1 *PON* gene family

The *PON* gene family consists of three members, *PON1*, *PON2*, and *PON3*, located adjacent to each other on the long arm of chromosome 7 between q21.3 and 22.1 in humans (117). The genes share considerable structural similarity and appear to have arisen by gene duplication from common evolutionary precursor. Within a given mammalian species, *PON1*, *PON2*, and *PON3* share approximately 60% identity at the amino acid level and about 70% identity at the nucleotide level (118). Between mammalian species, however, each of the three genes shares 79-90% identity at amino acid level and 81-89% identity on the nucleotide level (117, 119)). Codon 106 (lysine) present in *PON1* is missing in all *PON2* and *PON3* cDNA sequenced to date (117, 119).

2.2 PON1

PON1 is a calcium-dependent esterase consisting of 354 amino acids with a molecular mass of 45 kDa and in serum is exclusively located on HDL (27, 28). PON1 has an extremely hydrophobic N-terminal end that could anchor it to HDL (120); however, PON1 is not present in LDL or very low-density lipoprotein (VLDL), indicating a specific interaction with HDL by association with apo AI (35). As presented in figure 1, Draganov *et al.* summarized the available structure-function analysis of PON1 (121). This enzyme has two calcium binding sites, one of which is needed for its hydrolytic activity. While chelation of calcium inactivates PON1 activity and decreased its stability, it does not interfere with its ability to inhibit LDL oxidation (37, 122). PON1 has three cysteine residues: two forms an intramolecular disulfide linkage, while the free cysteine (residue 284) is required for optimal paraoxonase and arylesterase activities and is also essential for the action of PON1 in protecting LDL against oxidation (37, 122).

PON1 is synthesized primarily in the liver and a portion is secreted into the serum, where it is associated with HDL. PON1 activity is present in newborn and premature infant at about half the level found in adults (123). Adult levels are reached approximately 1 year after birth and appear to remain constant throughout life (123).

No differences between sexes in PON1 activity have been reported, eventhough PON1 is associated with HDL and there is an obvious difference in the overall circulating HDL concentration between men and women (123).

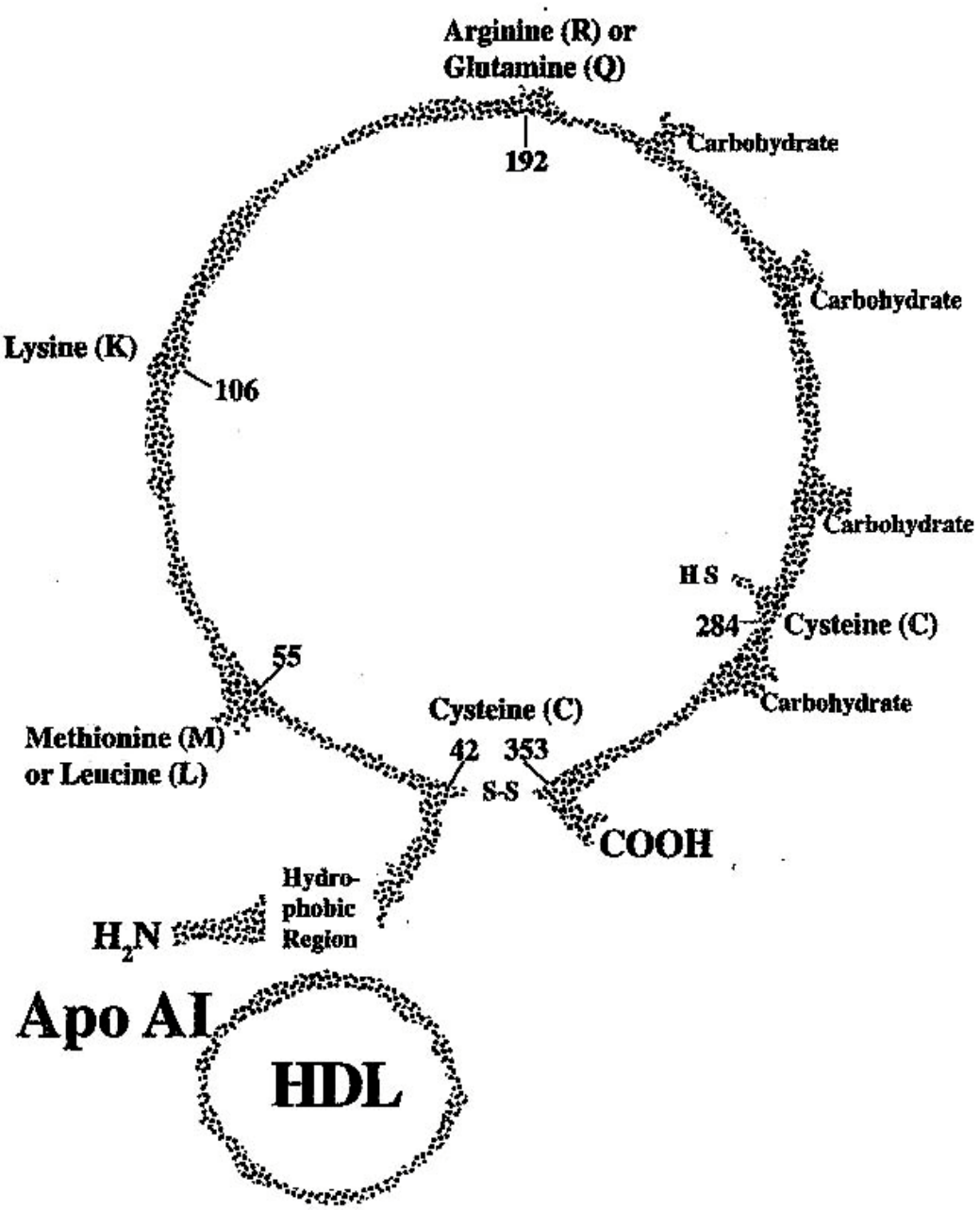

Figure 1. Human PON1 structure characteristics (119)

PON1 received its name from paraoxon, the toxic metabolite of insecticide parathion, which is one of its most studied substrate. In addition to paraoxon, PON1 has been shown to hydrolyze metabolites of number of other insecticides such as chlorpyrifos oxon and diazoxon, and also to detoxify various nerve agents such as sarin and soman (124). One natural physiological function of PON1 appears to be the metabolism of toxic oxidized lipids of both LDL particles as well as HDL particles. Mackness *et al.* were the first to demonstrate that purified human PON1 could inhibit LDL oxidation *in vitro* (23). Other studies have confirmed and extended this finding, demonstrating that PON1 both prevents the formation of oxidized LDL and inactivates LDL-derived oxidized phospholipids once they are formed (24-26). PON1 also protects phospholipids in HDL from oxidation (125). These actions suggest a role of PON1 in cardiovascular diseases and atherosclerosis.

2.2.1 Mechanisms for PON1 antiatherogenicity

PON1 protects HDL and LDL against oxidation

In vitro study, supplementation of human HDL or whole serum with purified PON1 significantly inhibits copper-induced lipoprotein oxidation in a concentration-dependent manner (125, 126).

PON1-specific, non-competitive inhibitors enhanced HDL oxidation induced by either copper or a free-radical-generating system (125).

Compared with oxidized HDL, HDL that was oxidized in the presence of PON1 increased cellular cholesterol efflux from macrophages. Both HDL-associated PON1 and purified PON1 significant protected human LDL from oxidation (125, 126).

PON1 hydrolyzes oxidized lipid in oxLDL

PON1 in HDL might protect against the induction of inflammatory responses in cells of the arterial wall by destroying biologically active lipids in mildly oxLDL (minimally modified LDL; MM-LDL) (25). Watson *et al.* have shown that purified PON1 can prevent the proinflammatory effects of oxLDL when incubated in a vascular cell coculture system (25). This was probably owing to the metabolism of

oxidized arachidonic acid derivatives in the sn-2 position of the LDL phospholipids (25, 122, 127). Searching for a possible mechanism for PON1-induced protection of lipoproteins (HDL and LDL) against oxidation revealed that PON1 can hydrolyze lipid peroxides in oxidized lipoproteins, with a major effect on oxidized lipoprotein associated cholesteryl linoleate hydroperoxides (127). Thus, HDL-associated PON1 esterase and peroxidase-like activity can contribute to the protective effect of PON1 against atherosclerosis by preventing lipoprotein oxidation products accumulation.

As shown in figure 2, Navab *et al.* have proposed the following sequence of events during the formation of MM-LDL containing the bioactive oxidized phospholipids (128). In step 1, LDL is seeded with reactive oxygen species (ROS). In step 2, LDL is trapped in the artery wall and receives further seeding molecules. In step 3, when a critical level of seeding molecules relative to phospholipids is reached in the LDL, a non-enzymatic oxidation process generates the active lipids. LDL may enter the subendothelial space, where it is seeded with ROS delivered from the artery wall cells (step 1). PON1 can prevent this step. While the cartoon depicts this as occurring in the subendothelial space, step 1 might actually occur in the microcirculation. If the LDL is seeded in the subendothelial space, it might remain there becoming trapped in the extracellular matrix, or the seeded LDL could exit into the circulation and re-enter the subendothelial space at another site where it would become trapped in the extracellular matrix. In step 2, the artery wall cells generate and transfer additional or different ROS to the trapped, seeded LDL. Presence of PON1 would inhibit the addition of ROS to the trapped LDL. The ROS transfer could occur within the cell, at the cell surface, or in an adjacent protected microdomain. Following this transfer of ROS to the seeded and trapped LDL, a non-enzymatic propagation of lipid oxidation occurs (step 3). These results in the formation of specific oxidized phospholipids which are present in MM-LDL and can induce nuclear factor kappa B (NF-κB) activation, monocyte binding, monocyte chemotactic protein 1 (MCP-1) production, and macrophage-colony stimulating factor (M-CSF) production and, as indicated, PON1 is capable of blocking each and every step in the formation of MM-LDL.

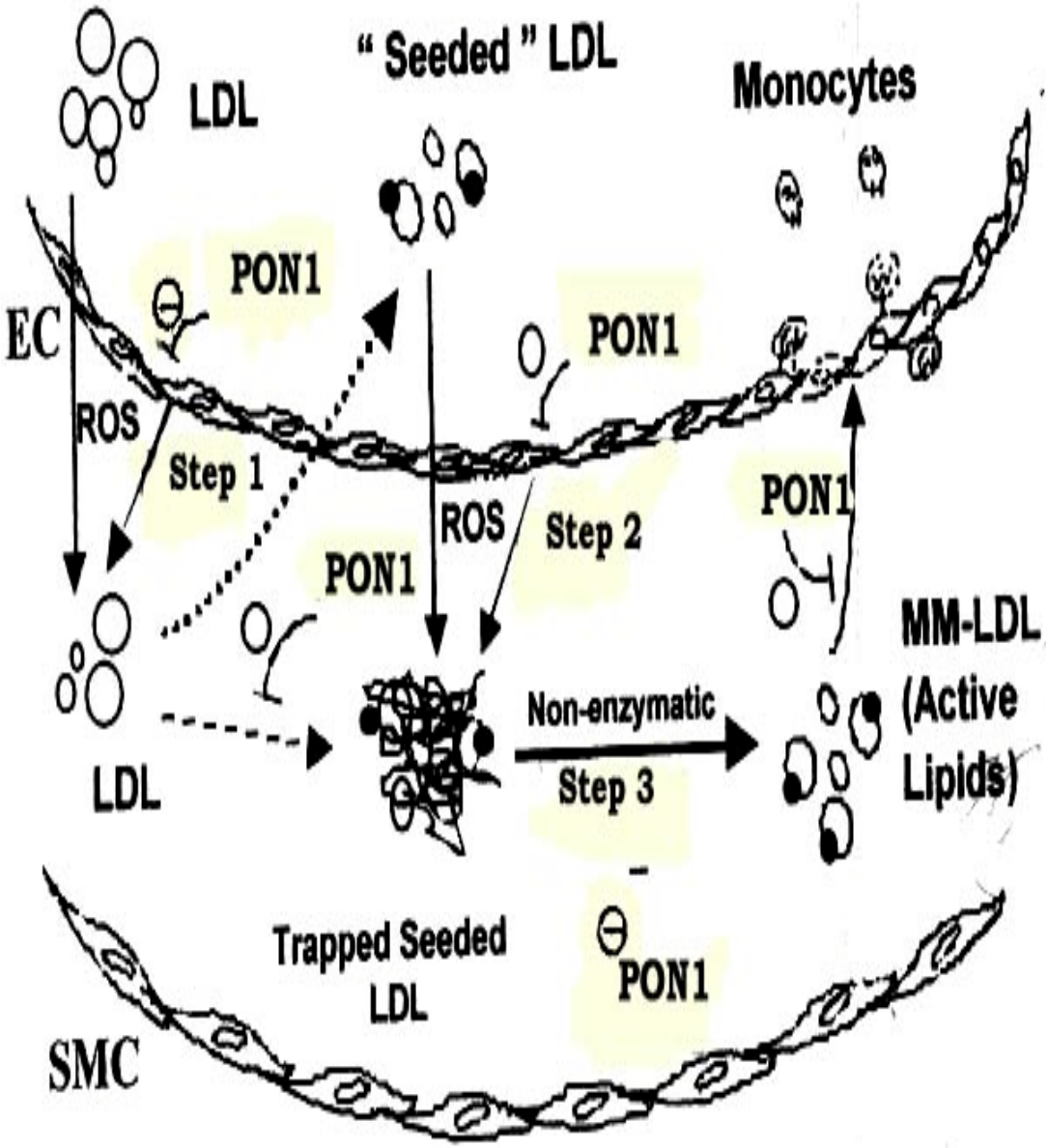

Figure 2. Three steps model for formation of mildly oxLDL and stages where PON1 can inhibit this process (128).

2.2.2 *PON1* polymorphisms

The *PON1* gene contains several polymorphisms in both the coding and the regulatory regions as demonstrated in figure 3.

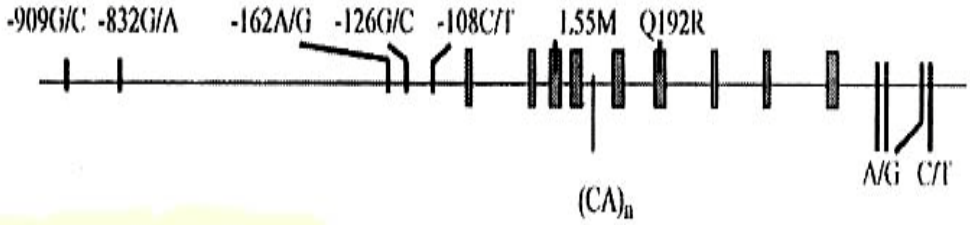

Figure 3. *PON1* polymorphisms (129)

Coding region polymorphisms

PON1 has two common coding polymorphisms, a leucine to methionine substitution at codon 55 (L55M) and a glutamine to arginine substitution at position 192 (Q192R) (33, 35). The L55M polymorphism has not been found to affect catalytic efficiency (33, 35) but has been associated with the variability of PON1 levels. $PON1_{M55}$ individuals were found to have lower levels of PON1 activity and PON1 mRNA (26, 42, 47). This effect seems to be related to linkage disequilibrium with various regulatory polymorphisms that determine on PON1 expression (129). The Q192R polymorphism has been found to affect the PON1 activity towards various substrates including paraoxon, diazoxon, soman and sarin (130). $PON1_{Q192}$ was found to hydrolyze paraoxon more slowly but diazoxon, sarin, and soman more rapidly than $PON1_{R192.}$ Phenylacetate hydrolysis rates are not polymorphic and both isoforms hydrolyze this substrate with equal rates (130).

Three new polymorphisms of the *PON1* gene, all associated with low PON1 activity, have been reported by Jarvik *et al.* (131). One polymorphism changes amino acid 194 from a tryptophan to produce a stop codon. The second is a proline/ leucine substitution of amino acid 90 which is only associated with the $PON1_{Q192}$ allele. The third polymorphism changes amino acid 124 from an aspartate to a missplice mutation, which is associated with the $PON1_{R192}$ allele. The frequency of these polymorphisms in the general population is unknown.

Regulatory region polymorphisms

Three groups have found polymorphisms in the regulatory region of *PON1* gene (47, 132, 133). Leviev and James found three *PON1* polymorphisms in a Swiss population (-107C/T, -824A/G, and -907G/C) (132). Suehiro *et al.*, studying a Japanese population, found *PON1* polymorphisms at -108C/T, -126G/C, and -160A/G (133). Brophy *at al*. found five *PON1* polymorphisms in a mostly Caucasian population (-108C/T, -126G/C, -162A/G, -823G/A, and -909G/C) (47). The numbering for all three groups begin at the base immediately preceding the translation start codon, although sequence variations among the group resulted in numbering differences. There are *PON1* polymorphisms at five positions in regulatory region, -107/-108, -126, -160/-162, -824/-832, and -907/-909.

Three different laboratories have reported somewhat different effects of the regulatory polymorphisms on PON1 expression. The effect of the -107C/T, -124A/G and -909G/C polymorphisms on PON1 expression was explored by Leviev and James (132). They used the Promega dual luciferase reporter gene assay to assess the effects of each base change on the transcriptional activity of the *PON1* promoter. They found that the -107C variant had approximately twice the activity as the -107T variant and the -824A variant had approximately 1.7 times the activity than -824G variant. Reporter gene assays did not show a difference in the efficiencies of the -907C/G variants. However, when PON1 levels were examined in individuals with the different regulatory polymorphisms, significant variation in PON1 levels was attributable to each polymorphism. The variants -107T, -824G and -907C were associated lower serum PON1 levels, while -107C, -824A and -907G were correlated with the highest PON1 concentrations and activities determined with the position 192 neutral substrate phenylacetate. Heterozygotes had intermediate PON1 levels. The -907C/G site appeared to have a minor contribution to transcription regulation.

Suehiro *et al.* reported the *PON1* -108C/T, -126G/C, and -160A/G polymorphisms (133). The luciferase reporter gene assay comparing the -108C with the -108T polymorphism demonstrated a 0.67-0.77 fold lower expression level from the -108T construct than seen with the -108C construct. These data correlated with PON1 levels in individuals processing $PON1_{-108CC}$ alleles who had about 1.2 fold

higher PON1 levels than the $PON1_{-108TT}$ individuals. They also noted linkage between the -126G/C and -160G/A polymorphisms.

Brophy *et al.* found that the *PON1* polymorphisms at position -909, -162 and -108 each had approximately a two-fold effect on PON1 expression levels (134). They noted that the effect was not strictly additive. A population study examined the effects of the -909, -162, -108, and 55 polymorphisms on expression level in 376 Caucasians (47). This study found that the *PON1* polymorphisms at -909 and 55 had little if any effect on *in vivo* expression of PON1. The polymorphism at -162 contributed a small amount (2.4%) and the -108 polymorphism contributed 22.4% of the variation in PON1 expression.

2.2.3 Association of *PON1* polymorphisms with CHD

Since it was proposed that PON1 could inhibit LDL oxidation, a large number of population association studies involving polymorphisms in the human *PON1* gene have been reported (135).

Case-control studies

Many groups have published clinical epidemiological studies of a case-control nature on the relationship of *PON1* polymorphisms to CHD presence (136). The majority have reported on the *PON1* Q192R polymorphism, presumably because of its capacity to affect the ability of PON1 to inhibit LDL oxidation (26). Fewer studies have been conducted on the *PON1* L55M coding region polymorphisms. Some have shown an association between the *PON1* 55L allele and atherosclerosis, but others have not (137). Of the many association studies with CHD, more than half yielded significant results. Moreover, in studies of the *PON1* Q192 R polymorphism, the 192R allele was consistently associated with CHD. A large meta-analysis of 43 genetic association studies involving more than 11,000 cases and 13,000 controls has found an association between $PON1_{R192}$ and CHD, while no significant association was found between $PON1_{M55}$ and the noncoding polymorphism -107T and CHD (136).

Some groups have shown that the *PON1* T-108C polymorphism in the regulatory region significantly affects PON1 levels, is a risk factor for CHD; however, this again has been disputed (135).

Prospective studies

The Norwick Park Prospective Heart Study II investigated *PON1* polymorphisms and CHD events (138). The study population was 3052 men ages 50 to 60 years and there were 205 documented CHD events at 6 years. The authors found no direct association with CHD for the *PON1* L55M and Q192R polymorphisms. However, some complex interactions were evident. The *PON1* L55M polymorphism increased the risk of CHD in smokers compared to nonsmokers.

In the lipoprotein and coronary Atherosclerosis Study, which was also prospective, the *PON1* Q192R polymorphism was not associated with severity, progression, or regression of coronary atherosclerosis, clinical events, or response to treatment with fluvastatin (139).

2.2.4 Association of PON1 activity and concentration with CHD

Case-control studies

Relative to genetic epidemiological studies, there have been few epidemiological studies that have included a measure of PON1 status, i.e., activity and concentration. The first conducted in 1985 indicated that PON1 activity was lower in people who had MI than in controls (140). Subsequently, Navab *et al.* showed that patients with high HDL-C but low PON1 were more susceptible to CHD than patients with low HDL-C but high PON1 (141); however, the number of subjects was small. In addition, Ayub *et al.* showed that PON1 activity and concentration were lower in people who had MI than in age- and gender-matched controls (142). Blood samples were taken within 2 hr of the onset of chest pain, strongly suggesting that PON1 activity was low before the event. The effect on PON1 activity was independent of the *PON1* Q192R genotype (142). The other large studies investigating the relationship of PON1 status and genotype, with cardiovascular disease (CVD) have been published (43, 143). All these studies found that PON1 status was associated with CVD, whereas the *PON1* genotypes were not. PON1 status was significantly lower in the populations with CVD, usually by up to 50% (43, 143). None of the *PON1* polymorphisms, whether considered singly or in combinations of haplotypes, were associated with CVD.

Prospective studies

The only prospective study of the relationship of PON1 status and CHD to report so far is the Caerphilly Prospective Study (144). The study included 1338 men ages 50-65 years who were studied for 10 years. PON1 activity towards paraoxon was 30% lower in men who had a new coronary event (n = 163) than in those who did not (n = 1175; p = 0.039). However, there were no differences in PON1 activity towards diazoxon or phenylacetate, PON1 concentration, or apo J concentration. There was a graded response of risk of new CHD events by quintile of paraoxon hydrolysis; men in the highest quintile of PON1 activity were 60% less likely to have a coronary event than those in the lowest quintile. PON1 activity predicted coronary events independent of all other coronary risk factors, including HDL (144). Low PON1 activity was a strong predictor of new events, in those in the highest quintile of risk, according to the Framingham equation, and in those who had documented CHD at entry into the study.

2.2.5 Modulation of PON1

The importance of PON1 in determining susceptibility or protection from LDL oxidation points to the relevance of factors affecting PON1 activity and levels of expression. Although genetic determinants, such as the *PON1* polymorphisms, play indeed a primary role in determining an individual's PON1 status, the contribution of other factors in modulating PON1 activity and concentration may also be important. Modulation of PON1 levels by several factors has been reported as follows:

Smoking (145)

Cigarette smoke extract was found to inhibit human PON1 activity (146). Compounds suggested to be responsible for inhibition of PON1 activity are various reactive aldehydes (acetaldehyde, formaldehyde, acrolein and crotonaldehyde), as well as aromatic hydrocarbon (146). Four studies in humans have confirmed that smoking is associated with reduced serum PON1 activity (147-150). The effect appears to reverse within a relatively short time (3-24 months), suggesting a

direct effect of cigarette smoke on PON1 activity, as indicated by the *in vitro* studies (146).

Alcohol (145)

An earlier *in vitro* study had indicated that human PON1 was inhibited by ethanol and other aliphatic alcohols (151), observations in animals and humans have shown that lower levels of ethanol increase PON1 activity and levels. Two studies in healthy middle-aged men and postmenopausal women showed an increase of PON1 activity following moderate alcohol consumption as wine, beer or spirits (152, 153). In another study, light drinkers had a 395% higher, whereas heavy drinkers had a 45% lower serum PON1 activity compared to non-drinkers (154). Similarly, rats fed low doses of ethanol showed 20-25% increase in serum and liver PON1 activity and a 59% increase in liver PON1 mRNA, while higher doses of ethanol caused a 25 and 51% decrease in serum and liver PON1 activity and liver PON1 mRNA levels, respectively (154). However, it should be noted that no association between alcohol consumption and serum PON1 activity were found in other human studies (147, 155).

Fat-rich diet (145)

Mice of the B6 strain fed an atherogenic diet (15.75% fat, 1.25% cholesterol) for 3 months showed an approximately 60% decrease in serum PON1 activity and a similar reduction in liver PON1 mRNA levels (156). On the contrary, in CH3 mice, both parameters were slightly increased, suggesting that even in mice genetic factors contribute to *PON1* gene expression. Indeed, hepatic PON1 mRNA levels were 50% lower in control CH3 mice compared to B6 mice (156).

Feeding of both normal New Zealand white rabbits and rabbits transgenic for human apo AI with an atherogenic diet (0.5% cholesterol) for 14 weeks, resulted in a 50% decrease in serum PON1 activity, with a partial recovery following 16 weeks of normal chow diet (157).

After consumption of a meal rich in used cooking fat, by a group of 12 healthy men, serum PON1 activity decreased by 27% for up to 8 hr, but returned to normal values by 12 hr (158).

Replacement of dietary saturated fat with trans fat was found to reduce serum PON1 activity in healthy men and women by about 6% (159).

Groups of rats were fed control diet supplemented with either triolein, tripalmitin or fish oil. The triolein diet significantly increased PON1 activity by 46%, while fish oil caused a significant decrease (-39%) in PON1 activity. The tripalmitin-enriched diet had no effect (160). These results suggest that the fatty acid composition of phospholipids may affect PON1 activity.

In a human study, in which 14 individuals with type 2 diabetes received meal rich in thermally stressed olive oil or safflower oil (161). Only the olive oil meal increased serum PON1 activity, and the effect was most pronounced in women (161).

Finally, administration of an omega-3 polyunsaturated fatty acid concentrate to a group of 14 patients with familial combined hyperlipidemia for 8 weeks, resulted in a modest (10%) but significant increase in PON1 concentration (162).

Antioxidants (145)

Various *in vitro* and *in vivo* studies in animals and humans have provided initial evidence that antioxidants can increase PON1 activity, possibly by protecting the enzyme from oxidative stress-induced inactivation. The antioxidant flavonoids quercitin and glabridin protected PON1 in micellar solution (isolated from other HDL components) from a loss of activity due to copper-induced oxidation (163). Similarly, serum PON1 levels in apo E deficient mice fed quercitin or red wine, increased by 113% and 75%, respectively, compared to controls (164).

Administration of pomegranate juice, which is rich in flavonoids with antioxidant activity, to apo E deficient mice, was shown to increase serum PON1 activity by 26-43% (165). Similarly, pomegranate juice consumption by a group of 13 healthy men resulted in a 20% increase in serum PON1 activity (166).

A study in 189 white men from the Pacific North West region of the United State found a positive correlation between the dietary and medicinal intakes of vitamin C and E and serum PON1 activity (148). However, two earlier studies in Finnish population reported that high intake of vegetables, possibly rich in vitamin C and E, was negatively correlated with serum PON1 activity (167, 168). No

associations between vitamin C and E and β-carotene intake and serum PON1 levels was reported in yet another study in 388 men and women in Spain (147).

Drugs

Most studies on the modulation of PON1 by pharmaceutical compounds have focused on lipid-lowering compounds. Such studies with statins and fibrates have yielded somewhat conflicting results. *In vitro* exposure of HuH7 human hepatoma cells to pravastatin, simvastatin and fluvastatin (10-100 mM) caused a 25-50% decrease in PON1 activity in the culture medium and a similar decrease in PON1 mRNA; both effects were reversed by mevalonate (169). In the same cells, fenofibric acid (250 mM) caused a 50 and 30% increase in PON1 activity and PON1 mRNA, respectively. Fenofibric acid was found to induced PON1 gene-promoter activity, while statins had an opposite effect (169).

The latter finding is in contrast with results obtained in hepatic human HepG2 cells, where simvastatin (1.5-2.5 μg/ml) was found to upregulate PON1 promoter activity (170).

In another *in vitro* study on isolated lipoproteins, two oxidized metabolites of atorvastatin (5-50 μM) and a metabolite of gemfibrozil (2-80 μM), but not the parent compounds, were found to increase HDL-associated PON1 activity (171).

A study in rats indicated that fluvastatin (20 mg/kg/day for 3 weeks) reduced both serum and liver PON1 activity, while a lower dose (2 mg/kg/day) was only effective towards liver activity. On the other hand, pravastatin (4 or 40 mg/kg/day for 3 weeks) was devoid of significant effects (172).

Studies in humans have provided similar contrasting results. An increase in serum PON1 activity was found in patients treated with simvastatin, gemfibrozil and micronized fenofibrate (63-65). Whereas, no changes in serum PON1 activity were reported by other studies in patients treated with simvastatin (173) ciprofibrate (67), bezafibrate and gemfibrozil (66).

2.3 PON2 (174)

PON2 is a widely expressed intracellular protein with a molecular mass of approximately 44 kDa (117, 175). While little is known about the physiologic or pathophysiologic role of this protein, PON2 has been reported to possess antioxidant properties. Stably transfected cells overexpressing PON2 exhibit significantly lower levels of intracellular oxidative stress when exposed to hydrogen peroxide or oxidized phospholipids (175). Rosenbalt *et al.* have also demonstrated that purified recombinant PON2 can protect against LDL oxidation (176). In addition, LDL incubated with stably transfected cells overexpressing PON2 has lower levels of lipid hydroperoxides, and is less able to induce monocyte transmigration through endothelial cells (175). Furthermore, PON2 is able to retard the oxidation of performed mildly oxidized LDL (175). Thus, one function of PON2 may be to act as a cellular antioxidant, protecting cells from oxidative stress. However, the mechanism by which PON2 is able to do this is unknown.

Similar to PON1, PON2 has several polymorphisms that are associated with a number of pathophysiologic conditions. Population studies have yielded a pair of amino acid substitutions with alanine or glycine at codon 148 (A/G148) and either cysteine or serine at codon 311 (C/S311). The A/G148 polymorphism has been associated with variations in TC and LDL-C levels, fasting plasma glucose levels. In addition, the polymorphism at position 311 has been associated with coronary artery disease, ischemic stroke in patients with type 2 diabetes mellitus (174).

2.4 PON3 (174)

PON3 is an approximately 40 kDa protein that associates with HDL in the circulation, albeit in much lower levels than PON1 (177, 178). Of the three proteins, PON3 is the most recently identified member and also the least characterized one. Similar to PON1 and PON2, PON3 also possess antioxidant properties. Draganov *et al.* have reported that rabbit PON3 purified from serum is capable of inhibiting copper-induced LDL oxidation *in vitro* (178). Reddy *et al.* have shown that LDL incubated with stably transfected cells overexpressing human PON3 has significantly less lipid hydroxide and is less able to induce monocyte chemotactic activity (177).

Furthermore, like PON1 and PON2, PON3 is able to retard biological effects of preformed mildly oxidized LDL (177).

In contrast to PON1 and PON2, no polymorphisms have been identified for PON3 until recently, when a serine-to-threonine substitution at codon 311 (S/T311) and a glycine to aspartic acid substitution at codon 324 (G/D324) were detected in a population in southern Italy (179). However, to date, the functional consequences of these two polymorphisms have not been reported.

3. Fenofibrate

Fenofibrate is a synthetic phenoxy-isobutyric acid derivative indicated for the treatment of dyslipidemia. A standard formulation of the drug was originally launched in 1975 (180). The bioavailability of the standard formulation was subsequently improved through a comicronisation process, leading to the development of micronized fenofibrate capsules, available in Europe since 1992. More recently, a new microcoated (suprabioavailable) 160-mg tablet formulation of micronized fenofibrate has been developed that has a superior pharmacokinetic profile to that of micronized fenofibrate 200 mg as shown in figure 4 (104).

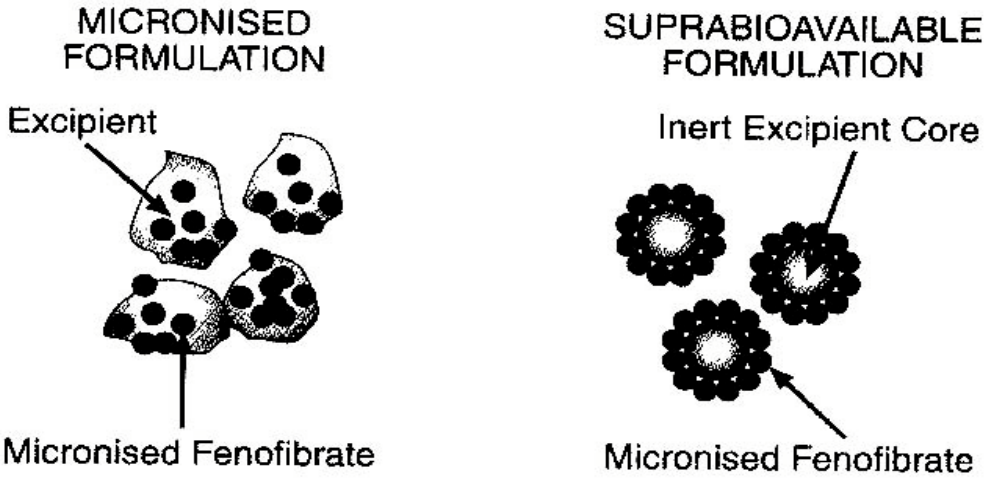

Figure 4. Micronized fenofibrate formulation (180)

3.1 Pharmacodynamic properties (101, 104, 181)

The lipid-modifying effects of fenofibrate are characterized by a reduction LDL-C and TC levels, a marked reduction in TG level and an increase in HDL-C levels.

Lipid-modifying effects of fenofibrate are mediated via activation of PPARs. The PPARs are ligand activated transcription factors that control gene expression by interacting with response elements located upstream from target genes (104, 181). Fenofibrate activates PPARα which regulates a number of genes involved in lipid metabolism including those encoding for apo CIII, apo AI, apo AII, and lipoprotein lipase (LPL) (101, 181). Fenofibrate-activated PPARα decreases the expression of apo CIII gene. This results in reduced apo CIII levels which in turn lead to increased LPL activity. Thus, the enhanced VLDL lipolysis together with increases in PPARα-mediated synthesis of apo AI and apo AII, augments the production of HDL and the rate of HDL-mediated RCT (101).

Modulation of the LDL subfraction distribution and promotion LDL catabolism via the LDL-receptor-mediated pathway are major features of the hypolipidemic effect of fenofibrate. Fenofibrate has been reported to produce favorable modulation of the LDL subfraction distribution, promoting the formation of larger, less dense LDL and reducing levels of dense LDL subfraction, in patients with combined dyslipidemia and in patients with type 2 diabetes mellitus (182).

Experimental studies have shown that fenofibrate has important anti-inflammatory effects on vascular cells, reducing the expression of adhesion molecules and proinflammatory cytokines (101).

Furthermore, fenofibrate has also demonstrated beneficial effects on Lp (a), apo B, fibrinogen and uric acid levels (104).

3.2 Pharmacokinetic properties (104, 180, 181)

Fenofibrate is a prodrug, which after absorption undergoes hydrolysis by tissue and plasma esterases to its principal active metabolite fenofibric acid (181).

In microcoated fenofibrate formulation, micronized fenofibrate is coated directly onto an inert excipient core (Figure 4). As a result of the micronized fenofibrate particles are immediately exposed to the dissolution medium and *in vitro*

dissolution after 10 min is increased by 46% over the non-microcoated micronized formulation (180). *In viv*o bioavailability is also increased and equivalent plasma levels of fenofibrate are obtained with lower doses of microcoated fenofibrate compared with non-microcoated micronized formulation. Microcoated fenofibrate 160 mg is considered to be bioequivalent to micronized fenofibrate 200 mg. The interindividual variation in maximum plasma concentration (Cmax) and the variation in the area under the plasma concentration time curve (AUC) associated with food intake were reduced with the microcoated formulation compared with the micronized formulation (180).

The Cmax of fenofibric acid occurs within 6 to 8 hr after fenofibrate administration and the absorption of fenofibrate is increased when administered with food. With the microcoated fenofibrate the extent absorption is increased by approximately 35% under fed as compared with fasting conditions (104). The fat content of a meal at the time of fenofibrate administration does not have a marked effect on pharmacokinetics (104)

Steady-state plasma concentrations of fenofibric acid are achieved after 5 days and the agent does not accumulate over time with repeated administration. At steady-state, the mean Cmax of fenofibric acid was 23 mg/L, and was reported approximately 5.4 hr after administration micronized fenofibrate 200 mg to healthy volunteers. Serum protein binding, mainly to albumin, exceeds 99% and is concentration-independent over the therapeutic dose range (183).

The half-life of fenofibric acid is 20 hr, allowing for once daily administration (104). Fenofibrate is excreted mainly in urine, primarily as fenofibric acid and fenofibric acid glucuronide. The total body clearance of micronized fenofibrate was 0.01 L/h/kg (104). The clearance is greatly reduced in patients with renal dysfunction and the dosage should be reduced in these patients.

3.3 Clinical efficacy (104)

The therapeutic efficacy of micronized fenofibrate 200 mg once daily has been evaluated in both comparative and noncomparative studies, and the therapeutic efficacy of microcoated fenofibrate 160 mg has been evaluated in two noncomparative studies in patients with type IIa, IIb or IV primary dyslipidemia.

3.3.1 Primary dyslipidemia (104)

Studies in patients with type IIa, IIb or IV primary dyslipidemia treated with micronized fenofibrate 200 mg or microcoated fenofibrate 160 mg once daily have reported reductions in TC levels of 12 to 30%. LDL-C levels were decreased in patients with type IIa, IIb dyslipidemia, generally by about 13-35% but underwent minimal change in patients with type IV dyslipidemia. There was a trend towards greater reductions in TG levels in patients with type IIb or IV (-32% to -53%) than in patients with type IIa dyslipidemia (-15% to -43%). Increase in HDL-C levels of up to 27% in patient with IIa dyslipidemia, of up to 34% in patient with IIb dyslipidemia, of up to 22% in patient with IV dyslipidemia were achieved (104). The increase in HDL-C levels achieved may depend on the baseline HDL-C levels in which greater percentage increases in HDL-C levels have been seen with lower baseline HDL-C levels (104) and may have contributed to the variation in the effect of fenofibrate on HDL-C seen between studies.

Noncomparative studies

Treatment with micronized fenofibrate 200 mg or microcoated fenofibrate 160 mg once daily for 2 to 6 months consistently reduced LDL-C (-0.3% to -34.7%), TC (+6% to -25%) and TG (-15% to -52%) levels, and increased HDL-C (+3% to +34.7%) levels (104). Furthermore, significant reductions from baseline in the TC to HDL-C ratio (-21.3% to -33%) were reported in micronized fenofibrate recipients with type IIa or IIb dyslipidemia (104). Beneficial long-term effects on TC, LDL-C, HDL-C and TG levels were also evident after 12 months of treatment with micronized fenofibrate 200 mg once daily (104).

In the trials differentiating between dyslipidemia subtypes, the greatest reduction in LDL-C levels occurred in patients with type IIa or IIb dyslipidemia, and patients with type IIb or IV dyslipidemia experienced the greatest reductions in TG levels (104).

Comparative studies

- **Comparisons with placebo**

Significantly greater reductions in LDL-C (-31.6% to -38.8% vs +0.5%), TC (-24.5% to -31.9% vs +0.5%) and TG (-26.7% to -40.8% vs +3.5%) levels, were reported in patients with type IIa or IIB dyslipidemia treated with micronized fenofibrate 200 to 400 mg daily than with placebo after 3 months of treatment (184). The percentage of patients with LDL-C levels < 3.36 mmol/L after 3-month treatment period was also higher among micronized fenofibrate than placebo (27.4% to 55.7% vs 0%).

- **Comparisons with HMG-CoA reductase inhibitors**

Greater reductions in TG levels were seen in patients with dyslipidemia who received micronized fenofibrate 200 mg once daily (-26% to -53%) than in recipients of atorvastatin 10 or 40 mg/day, simvastatin 10 or 20 mg/day or pravastatin 20 mg/day (-32.2% to +24.7%) (185-187). HDL-C levels also tended to increase to a greater extent in micronized fenofibrate than in HMG-CoA reductase inhibitor recipients (+1.3% to +33.6% vs -0.8% to +16.5%). Improvement in TC (-12.1% to -27.3%) and LDL-C (-7.7% to -33.5%) levels were seen in micronized fenofibrate recipients, although reductions in TC (-15.0% to -35.3%) and LDL-C (-17.0% to -38.6%) were usually greater in HMG-CoA reductase inhibitor recipients.

- **Comparisons with other fibrates**

Limited data are available comparing micronised fenofibrate with other fibrates. Greater improvements in lipid levels tended to be seen after administration of micronised fenofibrate than after administration of gemfibrozil or bezafibrate (104)

In combination with HMG-CoA reductase inhibitors

Combination therapy with micronized fenofibrate 200 mg once daily and fluvastatin 20 or 40 mg/day or atorvastatin 40 mg/day resulted in greater reduction in TC, LDL-C and TG levels than those observed with micronized fenofibrate alone (188, 189). Similar or greater changes in HDL-C levels were seen in combination therapy, compared with monotherapy.

3.3.2 Diabetic dyslipidemia

In Diabetes Atheroscelrosis Intervention Study (DAIS), significantly greater improvements from baseline in TC, LDL-C, HDL-C and TG levels were seen in patients with type 2 diabetes mellitus treated with micronized fenofibrate than in placebo group (190). In addition, micronized fenofibrate slowed the angiographic progression of focal coronary atherosclerosis. LDL peak diameter increased from baseline by a significant extent in micronized fenofibrate group but not in placebo group.

Similarly, in another double-blind, randomized, placebo-controlled study in 32 patients with type 2 diabetes mellitus, micronized fenofibrate was associated with significantly greater improvements in LDL-C, HDL-C, TC and TG levels than placebo. Moreover, as a consequence of its effect LDL-C and HDL-C, the TC to HDL-C ratio was reduced from baseline by a significantly greater extent in micronized fenofibrate group than in placebo group (-20% vs +10%) (191).

3.3.3 Metabolic syndrome

Treatment with micronized fenofibrate 200 mg once daily improved lipid levels and nonlipid parameters in a 12-week noncomparative study in 37 men with metabolic syndrome (192). After 12 week of treatment patients had significant reduction from baseline in mean levels of TC, LDL-C and TG and a significant increase from baseline in mean of HDL-C level. In addition, significant reductions from baseline in mean fibrinogen levels, uric acid levels, and mean factor VII activity were observed. Moreover, significant reduction from baseline in fasting serum insulin levels and insulin response during and oral glucose tolerance test and in systolic and diastolic blood pressure, were found.

3.4 Tolerability (104, 181)

Fenofibrate is generally well tolerated (104, 181). A post-marketing surveillance program involving 9884 patients with type IIa, IIb or IV dyslipidemia who were treated with micronized fenofibrate 200 mg once daily for 12 weeks showed that 3.8% of patients experienced adverse events (193). The discontinuation rate due to adverse events was 1.7%. The most frequently reported adverse events were those

affecting the gastrointestinal system (1.9% of patients) follow by adverse events associated with skin and appendages (0.6%), nervous system (0.4%), cardiovascular system (0.3%), and body as a whole (0.3%). Forty-three of 9884 patients (0.4%) experienced serious adverse events. Eight patients developed abnormal liver function tests and two patients experienced elevated levels of creatinine kinase (CK).

The tolerability of the microcoated fenofibrate 160 mg was assessed in two noncomperative 12-week trials (n = 177 and 198) (194). Adverse events were experienced by 24% and 40% of patients in the first study and in the second study, respectively. Twenty-two and thirty percent of adverse events were considered to be related to microcoated fenofibrate treatment in the first study and in the second study, respectively. However, none of the eight serious adverse events in these studies were considered drug-related. In both studies, few patients ($n \leq 3$) developed CK levels that were 4 to 5 or > 5 times the ULN, or alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels that were > 3 times the ULN.

CHAPTER III
MATERIALS AND METHODS

1. Materials

1.1 Chemicals and drug

Chemicals used in determination of PON1 activity

- Paraoxon [Sigma®, D9286]
- Phenylacetate [Sigma®, P2396]
- Diazinon-O-analogue [Chem Service®, West Chester, UK]
- Sodium chloride (NaCl) [Sigma®, S9625]
- Calcium chloride ($CaCl_2$) [Sigma®, C3881]
- Tris [hydroxymethyl] aminomethane and Tris hydrochloride (Trizma® Base) [Sigma®, T4753]

Chemicals used in determination of PON1 concentration

- Bovine serum albumin (BSA) [Sigma®, B2518]
- Sodium carbonate (Na_2CO_3) [Sigma®, S6139]
- Sodium bicarbonate ($NaHCO_3$) [Sigma®, S6014]
- Sodium chloride (NaCl) [Sigma®, S9625]
- Disodium phosphate (Na_2HPO_4) [Sigma®, S9763]
- Sodium acetate ($NaC_2H_3O_2$) [Sigma®, S2889]
- Citric acid ($C_6H_8O_7.H_2O$) [Sigma®, C1909]
- Potassium chloride (KCl) [Sigma®, P3911]
- Potassium dihydrogenphosphate (KH_2PO_4) [Anal R®]
- Polyclonal antibody (Paraoxonase IgG)
- Anti-rabbit IgG peroxidase conjugate [Sigma®, A0545]

- Tetramethylbenzidine (TMB) [Sigma®, T5525]
- Dimethyl sulfoxide (DMSO) [Sigma®, D8779]
- Sulphoric acid (H_2SO_4) [Anala R®]

Chemicals used in determination of *PON1* genotypes

- Ethylene diamine tetraacetic acid (EDTA) solution [Sigma®, E7889]
- Sodium dodecyl sulfate (SDS) solution [Sigma®, L4522]
- Ribonuclease (RNAase) [Sigma®, R5133]
- Proteinase K [Sigma®, P5568]
- Phenol [Sigma®, P4557]
- Ethanol (EtOH) [Anala R®]
- PCR core reagent kit [Roche®, USA]
- Tag DNA polymerase [Roche®, USA]
- Primers for amplification around $PON1_{55}$ position [Invitrogen®]

  Sense primer:

  5′ GAA GAG TGA TGT ATA GCC CCA G 3′

  Antisense primer:

  5′ TTT AAT CCA GAG CTA ATG AAA GCC 3′
- Primers for amplification around $PON1_{192}$ position [Invitrogen®]

  Sense primer:

  5′ TAT TGT TGC TGT GGG ACC TGA G 3′

  Antisense primer:

  5′ CAC GCT AAA CCC AAA TAC ATC TC 3′
- Primers for amplification around $PON1_{-108}$ position [Invitrogen®]

  Sense primer:

  5′ TAT TGT TGC TGT GGG ACC TGA G 3′

  Antisense primer:

  5′ CAC GCT AAA CCC AAA TAC ATC TC 3′
- Primers for amplification around $PON1_{-909}$ position [Invitrogen®]

  Sense primer:

5′ GAA GAG TGA TGT ATA GCC CCA G 3′

Antisense primer:

5′ TTT AAT CCA GAG CTA ATG AAA GCC 3′

- Restriction endonucleases [New England Biolabs®, Cambridge]

  NlaIII for $PON1_{55}$ position

  AlwI for $PON1_{192}$ position

  BstUI for $PON1_{-108}$ position

  BsmAI for $PON1_{-909}$ position

- Dimethyl sulfoxide (DMSO) [Sigma®, D8779]
- Sodium acetate buffer solution (CH_3COONa) [Sigma®, S2404]
- Tris borate EDTA buffer (TBE) [Sigma®, T7527]
- Agarose gel [GIBCO BRL®]
- Ethidium bromide (EtBr) [Sigma®, E8751]
- Gel loading solution [Sigma®, G2526]

Chemicals used in determination of lipid profiles

- Cholesterol kit [ABX diagnostics®, Shefford, UK]
- Triglycerides kit [ABX diagnostics®, Shefford, UK]
- HDL-C direct kit [ABX diagnostics®, Shefford, UK]
- Apo AI kit [ABX diagnostics®, Shefford, UK]
- Apo B kit [ABX diagnostics®, Shefford, UK]
- Oxidized LDL ELISA [Mercodia®, Uppsala, Sweden]
- Apo (a) ELISA [Mercodia®, Uppsala, Sweden]

Drug

- Microcoated fenofibrate (Supralip®) 160 mg [Laboratories FOURNIER S.A. France]

1.2 Instruments and supplies

The following equipments were used in this study.

- Cobas Mira autoanalyser [Roche®, Switzerland]

- Multiskan [Ascent Thermo Labsystem®]
- Microcentrifuge [Micro Centaur MSE®]
- Centrifuge [Beckman® and Kokusan®]
- Spectrophotometer [Beckman® Du68]
- PCR machine [GENIUS TECHNE®]
- Gel electrophoresis [GIBCO BRL®]
- UV transilluminator [Sigma® T2201]
- pH meter [Kent® EIL7020]
- Microwave [Sharp®]
- Water bath [Grant®]
- Automatic washer [Labsystem®]
- Microplate reader [Labsystem®]
- Shaker [Labsystem®]
- Freezer
- Magnetic stirrer
- Analytical balance
- Autopipettes 25, 200, 1000 μL
- Multichannel pipette 200 μL
- Microtiter plates
- Needles No 21
- Parafilm
- Volumetric flasks 100, 250, 1,000 mL
- Beakers 50, 100, 250, 500, 1,000 mL
- Cylinders 100, 250, 1,000 mL
- Disposable pipettes 3 mL
- Eppendorf tubes 1.5 mL
- Thermowell tubes 0.5 mL
- Plain tubes 7, 10 mL
- EDTA tubes 3 mL
- Test tubes 3 mL
- Polypropylene tubes 15 mL

2. Methods

The study was divided into 2 parts as follows:

Part 1: The study of PON1 status in healthy Thai population.

Part 2: The study of effect of fenofibrate therapy on PON1 status in patients with low HDL-C levels.

This research was studied in healthy Thai subjects with normal HDL-C levels and Thai patients with low HDL-C levels. All subjects who undergo medical check up at the division of Preventive Medicine, Royal Thai Air force, were screened before study entry for medical history, physical examination, and laboratory tests. Physical examination included weight, height and blood pressure measurements, chest X-ray, respiratory examination, abdominal examination, and eye examination. Laboratory tests included a complete blood count, blood urea nitrogen, creatinine, uric acid, fasting blood glucose (FBG), liver function tests, TC, TG, and HDL-C. A questionnaire was used to collect family and medical history, smoking and alcohol habits from the subjects. Data collection included weight, height, waist and hip circumferences, systolic and diastolic blood pressure (SBP and DBP) measurements using standard methods. Body mass index (BMI) was calculated as body weight (kg)/ height^2 (m^2). Waist hip ratio (WHR) was calculated as waist circumference (cm)/ hip circumference (cm).

The study was approved by the ethic committee of Bhumibol Adulyadej hospital and the committee on human rights related to human experimentation, Mahidol University, and the informed consents were obtained from all subjects.

2.1 Part 1: The study of PON1 status in healthy Thai population.

Null hypothesis

There is no *PON1* Q192R polymorphism in healthy Thai population.

Alternative hypothesis

There is *PON1* Q192R polymorphism in healthy Thai population.

2.1.1 Study design

This part was nondrug trial for study on PON1 status in healthy Thai population.

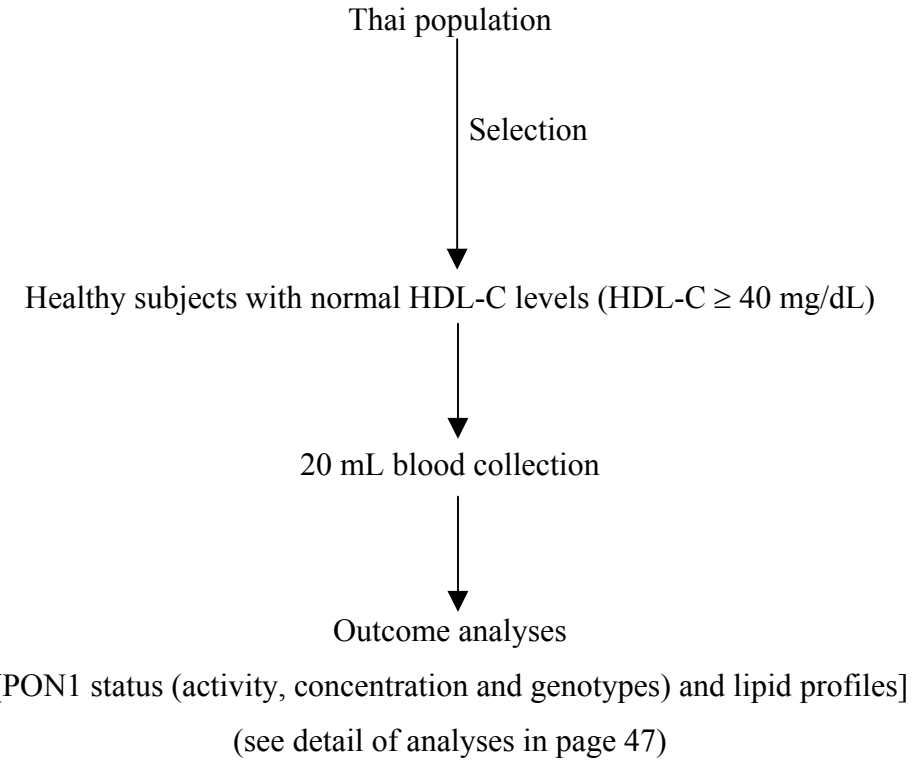

Figure 5. The study flow chart of PON1 status in healthy Thai population

2.1.2 Subjects

Subjects were healthy Thai population receiving no medication and having the following inclusion and exclusion criteria.

2.1.3 Inclusion criteria

- Gender : male or female
- Age : 18-70 years
- TC : < 240 mg/dL (6.20 mmol/L)
- TG : < 200 mg/dL (2.26 mmol/L)
- HDL-C : ≥ 40 mg/dL (1.03 mmol/L)
- Normal liver and renal functions
- Healthy person based on physical examination and laboratory tests (within normal range)
- Descent from Thai parents
- Voluntary participation by signing a consent form

2.1.4 Exclusion criteria

- Hypertension, diabetes mellitus, tuberculosis or thyroid disease
- History or physical signs of cardiovascular diseases or any cardiovascular disorders
- Inflammation, injury, or trauma in the previous month
- Pregnancy and breastfeeding
- Smoking history or alcohol abuse
- Taking concurrent medications known to affect lipid levels or antioxidant vitamins

2.1.5 Sample size calculation for study subjects (195)

Based on an allele is usually defined as polymorphic if it is present at a frequency of greater than 1% in the population.

Sample size (N) = $Z^2_{1-\alpha/2} \times \pi(1-\pi)/ e^2$

To set type I error of 5% $\rightarrow \alpha = 0.05$: two side test $\rightarrow Z_{1-\alpha/2} = 1.96$

π = genotypic frequency of 192RR = 1% (0.01)

e = standard error = 2% (0.02)

$N = 1.96^2 \times 0.01(1-0.01)/ (0.02)^2 = 95$

If genotypic frequency of 192RR was 1% (0.01), the totals of approximately 95 healthy subjects were required for this study.

2.2 Part 2: The study of effect of fenofibrate therapy on PON1 status in patients with low HDL-C levels.

Null Hypothesis

Fenofibrate therapy does not alter PON1 activity in patients with low HDL-C levels.

Alternative Hypothesis

Fenofibrate therapy increases PON1 activity in patients with low HDL-C levels.

2.2.1 Study design

This part was an open-label, noncomparative and intended to treat clinical trial. Microcoated fenofibrate [Supralip®] 160 mg/day treatment in dyslipidemic patients with low HDL-C levels was used. After a screening visit to determine possible eligibility, patients entered a 12-week dietary run-in period to establish baseline lipid levels. In the 12-week dietary run-in period, the patients were advised to practice diet control and regular exercise. At the end of this period, the eligible patients entered a 12-week active treatment period to receive microcoated fenofibrate 160 mg once daily with their breakfast for 12 weeks. The patients still continued to practice diet control and exercise throughout the study. The primary and secondary outcomes were measured at week 0, 6 and 12 after receiving medication. During each of the clinical visits (week 6 and 12), the patients were interviewed and physically examined for any possible side effects. Laboratory safety tests including CK, AST and ALT were also measured at week 0, 6 and 12. The drug would be discontinued if CK level was more than 5 times ULN or AST or ALT levels were more than 3 times ULN.

Patient adherences were assessed by counting the remaining drugs at each visit (week 6 and 12).

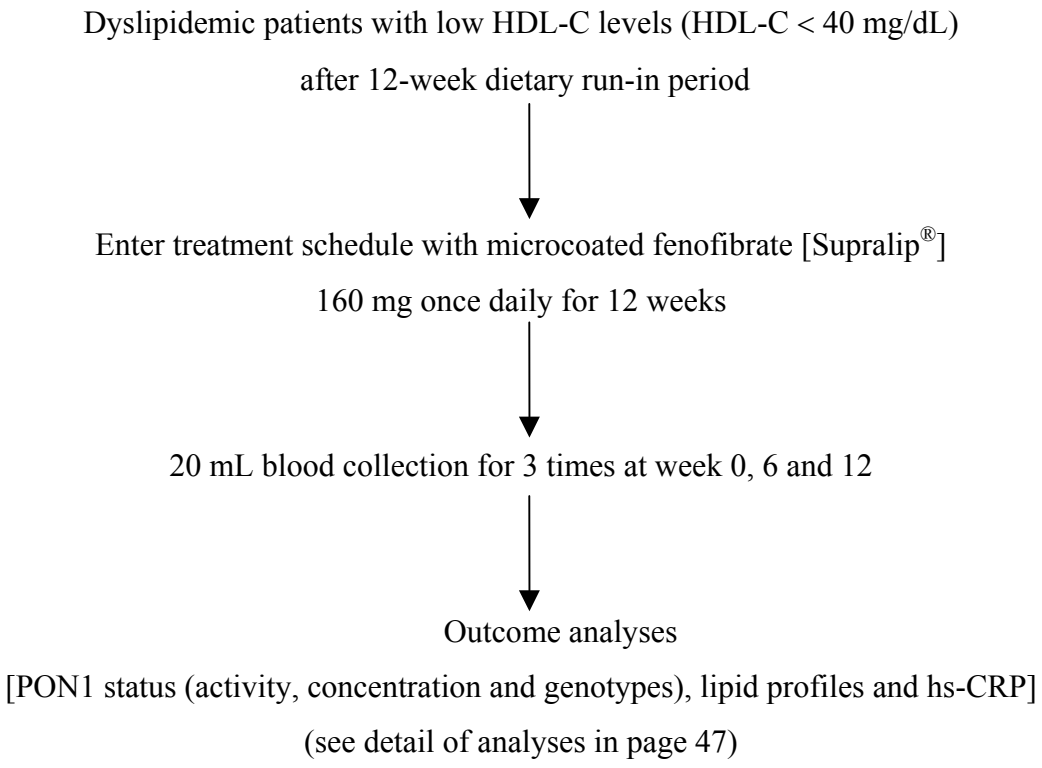

Figure 6. The study flow chart of effect of fenofibrate therapy on PON1 status in patients with low HDL-C levels

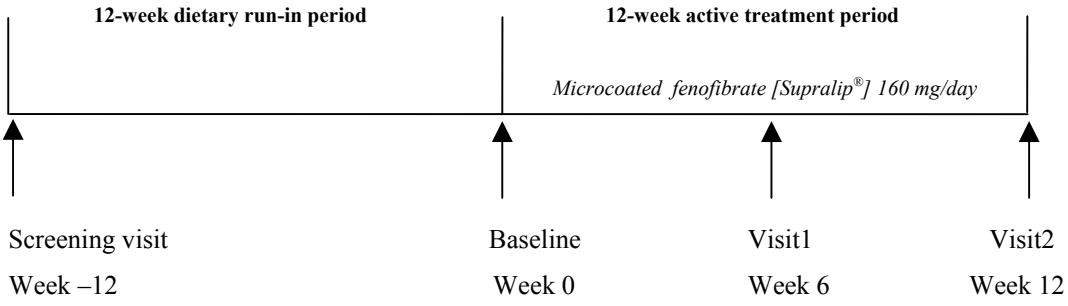

Figure 7. Treatment schedule in patients with low HDL-C levels

2.2.2 Study patients

Subjects were dyslipidemic patients with low HDL-C levels having the following inclusion and exclusion criteria.

2.2.3 Inclusion criteria

- Gender : male or female
- Age : 18-70 years
- TC : < 300 mg/dL (7.75 mmol/L)
- TG : < 400 mg/dL (4.51 mmol/L)
- HDL-C : < 40 mg/dL (1.03 mmol/L)
- Normal liver and renal functions
- Descent from Thai parents
- Voluntary participation by signing a consent form

2.2.4 Exclusion criteria

- Hypertension, diabetes mellitus, tuberculosis or thyroid disease
- History or physical signs of cardiovascular diseases or any cardiovascular disorders
- Inflammation, injury, or trauma in the previous month
- Pregnancy and breastfeeding
- Alcohol abuse
- Taking concurrent medications known to affect lipid levels or antioxidant vitamins
- Known hypersensitivity to fibrates

2.2.5 Sample size calculation for study patients (195)

Based on PON1 activity towards paraoxon between before and after antihyperlipidemic treatment (64)

Sample size (N) = $[(Z_\alpha+Z_\beta)^2 \times (Sd^2)] / (d^2)$

To set type I error of 5% $\rightarrow \alpha = 0.05$: one side test $\rightarrow Z_\alpha = 1.645$

To set type II error of 20% $\rightarrow \beta = 0.20$: 80% power $\rightarrow Z_\beta = 0.84$

Before treatment: PON1 activity (U/L) = 220 ± 98

After treatment: PON1 activity (U/L) = 253 ± 100

d = effect side = 253-220 = 33

Sd^2 = pooled variance = $SD_1^2 + SD_2^2 - 2r\ SD_1\ SD_2$

$= 100^2 + 98^2 - (2 \times 0.5 \times 100 \times 98) = 9804$

r = relative coefficient between PON1 activity towards paraoxon from before and after treatment = 0.5

$N = [(1.645+0.84)^2 \times (9804)] / (33)^2 = 55.59$

The totals of approximately 56 patients were required for this study.

2.3 Assessment of outcomes

The primary and secondary outcomes were assessed in the healthy subjects (part 1) for once and in the patients for 3 times at week 0, 6 and 12 after receiving medication (part 2).

2.3.1 Primary outcome

The primary outcome included:

1. PON1 status

   PON1 activity towards paraoxon

2.3.2 Secondary outcomes

The secondary outcomes included:

1. PON1 status
   - 1.1 PON1 activity towards phenylacetate and diazoxon
   - 1.2 PON1 concentration
   - 1.3 *PON1* genotypes

   PON1 genotypes for the *PON1* gene polymorphisms at coding region (L55M and Q192R) and regulatory region (T-108C and G-909C) were determined.
2. Lipid profiles
   - 2.1 TC
   - 2.2 TG
   - 2.3 HDL-C

2.4 LDL-C

2.5 Non-HDL-C

2.6 Apo AI

2.7 Apo B

2.8 OxLDL

2.9 Lp (a)

Lp (a) was assessed only in the healthy subjects (part 1).

3. Hs-CRP

Hs-CRP was assessed only in the patients with low HDL-C levels (part 2).

2.4 Blood sample collection

After a 12-hr overnight fast, venous blood was collected from the healthy subjects for once and the patients for 3 times at week 0, 6 and 12 after receiving medication. Twenty mL of blood was withdrawn via the needle No. 21. The 17 mL and 3 mL of blood were collected in the plain tubes and EDTA tube, respectively. Serum and plasma were prepared by centrifugation at 3,000 rpm, 4°C for 10 min. White cells were removed from the buffy coat of the EDTA tube for genomic DNA extraction. Serum, plasma and buffy coat were kept at -80°C for outcome analyses.

2.5 Determination of serum PON1 activity

The PON1 activity was determined by using paraoxon (O,O-diethyl-O-*p*-nitrophenyl phosphate), phenylacetate and diazoxon (diazinon-O-analog) as substrate (130, 196, 197).

2.5.1 Paraoxonase assay

Paraoxon hydrolytic activity was measured by adding 25 μL of serum to 500 μL of paraoxon solution (5.5 mM paraoxon in 100 mM Tris-HCl buffer containing 2 mM $CaCl_2$ at pH 8.0). The increase in optical density (OD) was recorded by continuous spectrophotometer at 405 nm, 25°C for 3 min.

Spontaneous hydrolysis of paraoxon was subtracted from total hydrolysis by determining a blank of the reaction mixture without serum.

Enzyme activity was calculated from the molar extinction coefficient (ε) of 17,100 M^{-1} cm^{-1} at 405 nm, pH 8.0.

$$\text{Activity} = \frac{(\text{OD/min}) \times \text{assay volume}}{\varepsilon \times \text{sample volume} \times \text{path length}}$$

A unit of paraoxon hydrolytic activity was defined as 1 nmol of 4-nitrophenol formed per min per mL.

2.5.2 Phenylacetate assay

Arylesterase activity was measured by adding 2.5 μL of serum to 100 μL of 13.62 mM phenylacetate solution and 900 μL of 100 mM Tris-HCl buffer containing 2 mM $CaCl_2$ at pH 8.0. The increase in OD was recorded by continuous spectrophotometer at 270 nm, 25°C for 2.5 min.

Spontaneous hydrolysis of phenylacetate was subtracted from total hydrolysis by determining a blank of the reaction mixture without serum.

Enzyme activity was calculated from the molar extinction coefficient (ε) of 1,310 M^{-1} cm^{-1} at 270 nm, pH 8.0.

$$\text{Activity} = \frac{(\text{OD/min}) \times \text{assay volume}}{\varepsilon \times \text{sample volume} \times \text{path length}}$$

A unit of arylesterase activity was defined as 1 μmol of phenylacetate hydrolyzed per min per mL.

2.5.3 Diazoxon assay

Diazoxon hydrolytic activity was measured by adding 5 μL of serum to 1,000 μL of diazinon-O-analogue solution (3.52 mM diazinon in 100 mM Tris-HCl and 2M NaCl buffer containing 2 mM $CaCl_2$ at pH 8.0). The increase in OD was recorded by continuous spectrophotometer at 270 nm, 25°C for 2.5 min.

Spontaneous hydrolysis of diazinon was subtracted from total hydrolysis by determining a blank of the reaction mixture without serum.

Enzyme activity was calculated from the molar extinction coefficient (ε) of 3,080 M^{-1} cm^{-1} at 270 nm, pH 8.0.

$$\text{Activity} = \frac{(\text{OD/min}) \times \text{assay volume}}{\varepsilon \times \text{sample volume} \times \text{path length}}$$

A unit of diazoxon hydrolytic activity was defined as 1 μmol of diazinon hydrolyzed per min per mL.

2.6 Determination of serum PON1 concentration

The serum PON1 concentration was determined by competitive ELISA using rabbit anti-human PON1 monospecific antibodies as reported in the previous study with some modifications (197, 202).

Preparation of reagents

1. Preparation of serum standards

   Prepared 1/10240 to 1/640 dilution of serum standards in 0.05 M carbonate buffer.
2. Preparation of serum samples

   Prepared 1/4000 dilution of serum samples in 0.05 M carbonate buffer.
3. Preparation of paraoxonase IgG (1/6400 dilution).

   Mixed 2 μL of paraoxonase IgG in 12.8 mL of 1% BSA in PBS.
4. Preparation of anti-rabbit IgG peroxide conjugate (1/2500 dilution).

   Mixed 10 μL of anti-rabbit IgG peroxide conjugate in 25 mL of 1% BSA in PBS.
5. Preparation of enzyme substrate.

   Dissolved 2 mg TMB in 200 μL of DMSO. Then mixed with 20 mL of 0.1 M citrate acetate buffer and 72 μL of urea hydrogen peroxide.

Procedures:

1. Added 100 μL of diluted serum standards and diluted serum samples to each well of microtiter plate.
2. Shook gently overnight at room temperature.
3. Washed once with 300 μL of 0.1% BSA in PBS.

4. Added 150 μL of 1% BSA in PBS and shook for 1 hr to block the remaining absorption sites.
5. Washed three times with 300 μL of 0.1% BSA in PBS.
6. Added 100 μL of paraoxonase IgG and shook for 1 hr.
7. Washed twice with 300 μL of 0.1% BSA in PBS.
8. Added 200 μL of anti-rabbit IgG peroxide conjugate and shook for 1 hr.
9. Washed three times with 300 μL of 0.1% BSA in PBS.
10. Added 200 μL of enzyme substrate solution quickly and shook for 15 min.
11. Added 50 μL of H_2SO_4 to stop reaction.
12. Read absorbance at OD 450 nm by using microtiter plate reader and calculated the result from standard curve.

2.7 Extraction of genomic DNA

Genomic DNA was extracted from white blood cells left at the interface of plasma after centrifugation by using phenol-ethanol method as following steps:

1. Prepared lysis buffer.

   One hundred mL of lysis buffer contained 1 mL of 0.5 M EDTA, 3 mL of 10% SDS, 1 mL of RNAase (10 mg/mL) and 1 mL of protinase K (10 mg/mL).
2. Added 3 mL of lysis buffer to buffy coat into 15 mL polypropylene tube.
3. Incubated in water bath at 65 °C for 3 hr.
4. Added 3 mL of phenol. Mixed gently then left for 15 min.
5. Centrifuged at 2,500 rpm for 10 min.
6. Carefully removed upper layer with pipette into new polypropylene tube.
7. Added 10 mL of 100% ethanol. Mixed gently by inversion.
8. Centrifuged at 3,000 rpm for 10 min.
9. Hooked out precipitation into eppendorf tube.
10. Added 100 μL of water. Resuspended the pellet by flicking.
11. Pipetted 10 μL of DNA into 0.5 mL thermowell tube.

The genomic DNA sample can now be used directly for PCR amplification.

2.8 Determination of *PON1* genotypes

PON1 genotypes for the *PON1* L55M, Q192R, T-108C and G-909C polymorphisms were determined by PCR amplification and restriction endonuclease enzyme digestion (47, 198).

2.8.1 PON 1_{55} DNA amplification

1. Prepared PCR reaction mixture.

   PCR reaction mixture contained 2,919 μL of water, 420 μL of 10x buffer, 252 μL of $MgCl_2$, 21 μL of each deoxyribonucleotide triphosphate (dNTP), 30 μL of each primer and 21 μL of Tag DNA polymerase.
2. Added 90 μL of PCR reaction mixture to 10 μL of each DNA sample.
3. PCR cycling was carried out in PCR machine as following conditions:

   Denatured DNA at 95 °C for 5 min 1 cycle

   Then 50 cycles Denatured at 94 °C for 1 min

   Annealed at 61 °C for 30 sec

   Extended at 72 °C for 1 min

   Then 1 cycle at 72 °C for 7 min

Restriction endonuclease digestion of PCR amplified products

1. Added 40 μL of 3 M sodium acetate and 500 μL of 100% ethanol to 100 μL of each PCR product.
2. Mixed and left overnight to precipitate at -80 °C.
3. Span samples at 10,000 rpm for 20 min at room temperature.
4. Discarded supernatant.
5. Digested with 20 μL of NlaIII overnight at 37 °C.
6. Added 2 μL of gel loading solution and mixed.
7. Ran 20 μL of samples on 3% agarose gel with EtBr at 80 mA for 2.5 hr.

8. Visualized DNA on UV transilluminator.
   NlaIII cut MM genotype into 44 base pair (bp) and 126 bp but did not cut LL genotype.

2.8.2 PON 1_{192} DNA amplification

1. Prepared PCR reaction mixture.
   PCR reaction mixture contained 2,919 μL of water, 420 μL of 10x buffer, 252 μL of $MgCl_2$, 21 μL of each dNTP, 30 μL of each primer and 21 μL of Tag DNA polymerase.
2. Added 90 μL of PCR reaction mixture to 10 μL of each DNA sample.
3. PCR cycling was carried out in PCR machine as following conditions:

   Denatured DNA at 95 °C for 5 min 1 cycle
   Then 50 cycles Denatured at 94 °C for 1 min
   Annealed at 61 °C for 30 sec
   Extended at 72 °C for 1 min
   Then 1 cycle at 72 °C for 7 min

Restriction endonuclease digestion of PCR amplified products

1. Added 40 μL of 3 M sodium acetate and 500 μL of 100% ethanol to 100 μL of each PCR product.
2. Mixed and left overnight to precipitate at -80 °C.
3. Span samples at 10,000 rpm for 20 min at room temperature.
4. Discarded supernatant.
5. Digested with 20 μL of AlwI overnight at 37 °C.
6. Added 2 μL of gel loading solution and mixed.
7. Ran 20 μL of samples on 3% agarose gel with EtBr at 80 mA for 2.5 hr.
8. Visualized DNA on UV transilluminator.

AlwI cut RR genotype into 33 bp and 66 bp but did not cut QQ genotype.

2.8.3 PON 1_{-108} DNA amplification

1. Prepared PCR reaction mixture.

   PCR reaction mixture contained 2,736 μL of water, 420 μL of 10x buffer, 252 μL of $MgCl_2$, 183 μL of DMSO, 21 μL of each dNTP, 21 μL of each primer and 21 μL of Tag DNA polymerase.
2. Added 90 μL of PCR reaction mixture to each 10 μL of each DNA sample.
3. PCR cycling was carried out in PCR machine as following conditions:

   Denatured DNA at 95 °C for 5 min 1 cycle

   Then 60 cycles Denatured at 94 °C for 1 min
   Annealed at 61 °C for 30 sec
   Extended at 72 °C for 1 min

   Then 1 cycle at 72 °C for 7 min

Restriction endonuclease digestion of PCR amplified products

1. Added 40 μL of 3 M sodium acetate and 500 μL of 100% ethanol to 100 μ L of each PCR product.
2. Mixed and left overnight to precipitate at -80 °C.
3. Span samples at 10,000 rpm for 20 min at room temperature.
4. Discarded supernatant.
5. Digested with 20 μL of BstUI overnight at 60 °C.
6. Added 2 μL of gel loading solution and mixed.
7. Ran 20 μL of samples on 3% agarose gel with EtBr at 80 mA for 2.5 hr.
8. Visualized DNA on UV transilluminator.

   BstUI cut CC genotype into 52 bp and 76 bp but did not cut TT genotype.

2.8.4 PON 1_{-909} DNA amplification

1. Prepared PCR reaction mixture.

   PCR reaction mixture contained 2,736 μL of water, 420 μL of 10x buffer, 252 μL of $MgCl_2$, 183 μL of DMSO, 21 μL of each dNTP, 21 μL of each primer and 21 μL of Tag DNA polymerase.

2. Added 90 μL of PCR reaction mixture to each 10 μL of each DNA sample.
3. PCR cycling was carried out in PCR machine as following conditions:

   Denatured DNA at 95 °C for 5 min 1 cycle

   Then 60 cycles Denatured at 94 °C for 1 min

   Annealed at 61 °C for 30 sec

   Extended at 72 °C for 1 min

   Then 1 cycle at 72 °C for 7 min

Restriction endonuclease digestion of PCR amplified products

1. Added 40 μL of 3 M sodium acetate and 500 μL of 100% ethanol to 100 μL of each PCR product.
2. Mixed and left overnight to precipitate at -80 °C.
3. Span samples at 10,000 rpm for 20 min at room temperature.
4. Discarded supernatant.
5. Digested with 20 μL of BsmAI overnight at 55 °C.
6. Added 2 μL of gel loading solution and mixed.
7. Ran 20 μL of samples on 3% agarose gel with EtBr at 80 mA for 2.5 hr.
8. Visualized DNA on UV transilluminator.

   BsmAI cut GG genotype into 50 bp and 206 bp but did not cut CC genotype.

2.9 Determination of serum cholesterol

Cholesterol concentration in serum was determined by enzyme colorimetric test, cholesterol oxidase/ phenol 4-aminoantipyrine (CHOD/PAP) method by using ABX diagnostics cholesterol kit. Three µL of serum samples, cholesterol controls and cholesterol standards were mixed with 280 µL of reagent which consisted of enzyme reagent and buffer solution. The mixtures were incubated at 37°C for 5 min, thereafter, they were measured absorbance at 500 nm against reagent blank within 30 min by using Cobas Mira autoanalyser. Serum cholesterol was calculated by using standard curve.

2.10 Determination of serum triglycerides

Triglycerides in serum were determined by enzyme colorimetric test, glycerol 3 phosphate oxidase/ phenol 4-aminoantipyrine (GPO/PAP) method by using ABX diagnostics triglycerides kit. Three µL of serum samples, triglyceride controls and triglyceride standards were mixed with 290 µL of reagent which consisted of enzyme reagent and buffer solution. The mixtures were incubated at 37°C for 5 min, thereafter, they were measured absorbance at 500 nm against reagent blank within 30 min by using Cobas Mira autoanalyser. Serum triglyceride was calculated by using standard curve.

2.11 Determination of serum HDL-C direct

The 2.4 µL of serum samples, HDL-C controls, and HDL-C standards were mixed with 240 µL of reagent 1 (polyanion) and 80 µL of reagent 2 (enzyme reagent). The mixtures were incubated at 37°C for 5 min, thereafter, they were measured absorbance at 500 nm against reagent blank within 30 min by using Cobas Mira autoanalyser. Serum cholesterol was calculated by using standard curve.

2.12 Determination of serum LDL-C

LDL-C concentration in the part 1 of study was determined by using of Friedewald equation (199),

LDL-C (mg/dL) = total cholesterol - HDL-C - triglycerides/5*

(* 2.2 if units were mmol/L)

2.13 Determination of serum LDL-C direct

LDL-C concentration in the part 2 of study was determined at Ramathibodi hospital by using Dade Behring LDL-C direct kit.

2.14 Determination of serum non-HDL-C

Serum non-HDL-C was calculated by subtracting HDL-C from TC (11)

2.15 Determination of serum apo AI

Apo AI concentration in serum was determined by using ABX Diagnostics apo AI kit. Seven μL of serum samples, apo AI controls and apo AI standards were mixed with 60 μL of apo AI antibody reagent, 23.3 μL of accelerator reagent and 106.7 μL of sample diluent. The mixtures were incubated at 37°C for 5 min, thereafter, the resulting immune complexes were measured by turbidimetric technique at 340 nm against reagent blank within 30 min by using Cobas Mira autoanalyser. Serum apo AI was calculated by using standard curve.

2.16 Determination of serum apo B

Apo B concentration in serum was determined by using ABX Diagnostics apo B kit. Seven μL of serum samples, apo B controls and apo B standards were mixed with 200 μL of apo B antibody reagent, 16.7 μL of accelerator reagent and 83.3 μL of sample diluent. The mixtures were incubated at 37°C for 5 min, thereafter, the resulting immune complexes were measured by turbidimetric technique at 340 nm against reagent blank within 30 min by using Cobas Mira autoanalyser. Serum apo B was calculated by using standard curve.

2.17 Determination of plasma oxLDL

Plasma oxLDL was measured by using a solid phase two-site enzyme immunoassay (ELISA). It was based on the directed against separate antigenic determinants on the oxidized apo B molecule. During incubation oxLDL in the sample reacted with anti-oxLDL antibodies bound to microtitration well. After washing, that removed non-reactive plasma components, a peroxidase conjugated anti-apo B antibody recognized the oxLDL bound to the solid phase. After a second incubation

and a simple washing step that removed unbound enzyme labeled antibody, the bound conjugate was detected by reaction with TMB. The reaction was stopped by adding acid to give a colorimetric endpoint that was read spectrophotometrically at 450 nm.

Preparation of reagents

1. Preparation of samples.

   Added 2,000 µL of sample buffer in 25 µL of plasma sample and mixed to give 1/81 dilution of sample. Then, added 2,000 µL of sample buffer in 25 µL of 1/81 dilution of sample and mixed to give 1/6,561 dilution of sample.
2. Preparation of standards and controls.

   Added 1,000 µL of redistilled water per vial of each standard and control and mixed.
3. Preparation of conjugate solution.

   Prepared the conjugate solution by mixing 1,200 µL of conjugate stock solution with 12 mL of conjugate buffer.
4. Preparation of washing solution.

   Added 40 mL of washing solution concentrate to 800 mL of redistilled water and mixed.

Procedures:

1. Added 25 µL of standards/ controls/ diluted samples to anti-oxidized LDL wells.
2. Added 100 µL of assay buffer.
3. Incubated on a shaker for 2 hr at room temperature.
4. Washed 6 times with washing solution by using automatic washer.
5. Added 100 µL of conjugate solution.
6. Incubated on a shaker for 1 hr at room temperature.
7. Washed 6 times with automatic washer.
8. Added 200 µL of peroxidase substrate.
9. Incubated for 15 min at room temperature, no shaking.

10. Added 50 μL of stop solution.
11. Measured the absorbance at 450 nm by using multiskan and calculated the results from standard curve.

2.18 Determination of serum Lp (a)

Serum apo (a) was measured by using a solid phase two-site enzyme immunoassay (ELISA). It was based on the direct sandwich technique in which two monoclonal antibodies were directed against separate antigenic determinants on the apo (a) molecule. During incubation apo (a) in the sample reacted with peroxidase-conjugated anti-apo (a) antibodies and anti-apo (a) antibodies bound to microtitration well. A simple washing step removed unbound enzyme labelled antibody. The bound conjugate was detected by reaction with TMB. The reaction was stopped by adding acid to give a colorimetric endpoint that was read spectrophotometrically at 450 nm.

Preparation of reagents

1. Preparation of sample diluent.

   Mixed 1 tablet of sample diluent tablet with 2.5 mL of stabilization solution and added to 250 mL of redistilled water.
2. Preparation of standards and controls.

   Added 500 μL of redistilled water per vial of each standard and control and mixed.
3. Preparation of samples.

   Added 25 μL of pretreatment solution in 25 μL of serum sample and control. Mixed and incubated for 1 hr at room temperature. Then, added 1.5 mL of sample diluent and mixed to give 1/62 dilution of sample. Then, added 0.6 mL of sample diluent to 0.2 mL of 1/62 dilution of sample and mixed to give 1/248 dilution of sample.
4. Preparation of conjugate solution.

   Prepared the conjugate solution by mixing 300 μL of conjugate stock solution with 6 mL of conjugate buffer.
5. Preparation of washing solution.

Added 40 mL of washing solution concentrate to 800 mL of redistilled water.

Procedures:

1. Added 25 μL of standards/ pretreated samples/ pretreated controls to anti-apo (a) wells.
2. Added 50 μL of conjugate solution.
3. Incubated on a shaker for 1 hr at room temperature.
4. Washed 4 times with washing solution by using automatic washer.
5. Added 200 μL of peroxidase substrate.
6. Incubated for 15 min at room temperature.
7. Added 50 μL of stop solution.
8. Measured the absorbance at 450 nm by using multiskan and calculated the results from standard curve.
   The concentration of apo (a) was expressed as Units/L.
   Unit of apo (a) was approximately equal to 0.7 mg Lp (a) protein.

2.19 Determination of serum hs-CRP

CRP concentration in serum was determined at Ramathibodi Hospital by immunonephelometry using Dade Behring hs-CRP.

2.20 Statistic analysis

The continuous parameters were expressed as means ± standard deviation (SD) if they had a normal distribution and the student's unpaired t-test was used for comparisons. The parameters with non-normal distribution were expressed as median (range) and the Mann-Whitney U test was used to compare the difference.

The normality of the sample distribution of each continuous parameter was tested with the Kolmogorov-Smirnov test.

Allele frequencies were calculated by the gene counting method. The Chi-square test was used to determine both the observed genotype frequencies deviated from Hardy-Weinberg equilibrium expectations and to evaluate the significance of the linkage disequilibrium between each polymorphism pair.

One-way ANOVA and Kruskal Wallis tests were used for comparison of normally and non-normally distributed parameters among genotypes, respectively.

Changes from baseline outcomes were analyzed using the Student's paired t-test for outcomes with the normal distribution and the Wilcoxon test for outcomes with the non-normal distribution.

Pearson and Spearman correlation tests were used to test the strength of associations of parameters with normal distribution and non-normal distribution, respectively.

Parameters influencing serum PON1 concentration and activity were analyzed by using stepwise multiple regression analysis.

A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed by using SPSS version 11 for Windows software.

CHAPTER IV
RESULTS

The results of this study were divided into 2 parts as follows:

Part 1: The study of PON1 status in healthy Thai population

Part 2: The study of effect of fenofibrate therapy on PON1 status in patients with low HDL-C levels

1. Part 1: The study of PON1 status in healthy Thai population

A total of 202 subjects were originally screened. One-hundred and thirty-four subjects were eligible for entering the part 1 of study.

1.1 Demographic characteristics of healthy subjects

The demographic details of healthy subjects are presented in table 3. The mean age, BMI, WHR, SBP, DBP and FBG were 35.9 years, 22.3 kg/m^2, 0.78, 121.4 mmHg, 70.0 mmHg, and 4.9 mmol/L, respectively. The study population had a greater proportion of males (males = 100, female = 34). Compared with females, males were significantly younger (33.7 vs 42.4 years; $p < 0.001$) and had significantly higher WHR (0.80 vs 0.72; $p < 0.001$). There were no significant differences in mean BMI (22.5 vs 21.8 kg/m^2), SBP (122.4 vs 118.4 mmHg), DBP (70.6 vs 68.4 mmHg), and FBG (4.8 vs 4.9 mmol/L) between males and females.

1.2 Lipoprotein profiles of healthy subjects

As shown in table 3, the average values of TC, TG, HDL-C, LDL-C and non-HDL-C levels in these healthy Thais were 5.27, 0.76, 1.41, 3.47, and 3.86 mmol/L, respectively. The values of Lp (a) and oxLDL levels were 13.77 mg/dL and 35.03 U/L, respectively. The mean concentrations of apo AI and apo B were 1.36 and 0.91 g/L. There was no significant difference in the levels of TC, TG, LDL-C, non-HDL-C, oxLDL or apo B concentrations between males and females. However, the levels of

HDL-C, apo AI and Lp (a) were significantly higher in females than in males (1.54 vs 1.37 mmol/L; $p < 0.001$, 1.41 vs 1.34 g/L; $p < 0.05$, 20.70 vs 11.61 mg/dL; $p < 0.01$, respectively).

Table 3. Baseline characteristics of healthy subjects

Parameters	Male (n = 100)	Female (n = 34)	Total (n = 134)
Age (years)	33.7 ± 10.1	42.4 ± 7.2***	35.9 ± 10.2
BMI (kg/m^2)	22.5 ± 2.6	21.8 ± 2.6	22.3 ± 2.6
WHR	0.80 ± 0.04	0.72 ± 0.04 ***	0.78 ± 0.05
SBP (mmHg)	122.4 ± 9.3	118.4 ± 12.4	121.4 ± 10.3
DBP (mmHg)	70.6 ± 8.1	68.4 ± 9.3	70.0 ± 8.4
FBG (mmol/L)	4.8 ± 0.5	4.9 ± 0.4	4.9 ± 0.5
TC (mmol/L)	5.26 ± 0.67	5.31 ± 0.59	5.27 ± 0.65
TG (mmol/L)[a]	0.77 (0.34-2.22)	0.72 (0.41-1.79)	0.76 (0.34-2.22)
HDL-C (mmol/L)	1.37 ± 0.23	1.54 ± 0.22 ***	1.41 ± 0.24
LDL-C (mmol/L)	3.49 ± 0.67	3.42 ± 0.55	3.47 ± 0.64
Non-HDL-C (mmol/L)	3.89 ± 0.70	3.78 ± 0.57	3.86 ± 0.67
Lp (a) (mg/dL)[a]	11.61 (0.65-68.77)	20.70 (2.39-62.56)**	13.77 (0.65-68.77)
OxLDL (U/L)	35.21 ± 11.50	34.51 ± 9.90	35.03 ± 11.08
Apo AI (g/L)	1.34 ± 0.17	1.41 ± 0.15*	1.36 ± 0.17
Apo B (g/L)	0.92 ± 0.17	0.88 ± 0.13	0.91 ± 0.16

Values are shown as mean ± SD. [a]Values are shown as median (range).

Significant differences between results for males and females are given:

*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

1.3 Serum PON1 concentration and activity in healthy subjects

Table 4 demonstrates serum PON1 concentration and activity towards the three substrates. The mean of PON1 concentration was 87.6 μg/mL. The median values of PON1 activity towards paraoxon, phenylacetate and diazoxon were 271.8 nmol/min/mL, 74.6 μmol/min/mL, and 9.5 μmol/min/mL, respectively. When the PON1 levels were expressed by gender, it was observed that PON1 concentration was significantly higher in females than males (96.5 vs 84.6 μg/mL; $p < 0.05$). There were no significant differences in PON1 activity towards the three substrates between males and females although the median values for serum PON1 activity towards paraoxon and phenylacetate tended to be higher in females (304.4 vs 264.5 nmol/min/mL and 76.5 vs 74.3 μmol/min/mL).

Table 4. PON1 concentration and activity towards three substrates of healthy subjects

PON1 levels	Male	Female	Total
Concentration (μg/mL)	84.6 ± 23.1	96.5 ± 24.3*	87.6 ± 23.9
Paraoxon hydrolysis (nmol/min/mL)[a]	264.5 (55.5-453.1)	304.4 (107.5-486.2)	271.8 (55.5-486.2)
Phenylacetate hydrolysis (μmol/min/mL)[a]	74.3 (42.1-103.6)	76.5 (26.9-100.3)	74.6 (26.9-103.6)
Diazoxon hydrolysis (μmol/min/mL)[a]	10.1 (1.2-21.3)	8.2 (3.9-21.4)	9.5 (1.2-21.4)

Values are shown as mean ± SD. [a]Values are shown as median (range).

Significant differences between results for males and females are given: *$p < 0.05$.

1.4 Correlations of various lipid parameters

Table 5 and figure 8 to 25 summarize the correlation coefficients of various lipid parameters. HDL-C was negatively correlated with TG (r = -0.255; p = 0.003; figure 8), LDL-C (r = -0.195; p = 0.024; figure 9), non-HDL-C (r = -0.239; p = 0.005; figure 10), oxLDL (r = -0.283; p = 0.001; figure 11), and apo B (r = -0.240; p = 0.005; figure 12), and positively correlated with apo AI (r = 0.869; $p < 0.001$; figure 13).

LDL-C had positive correlation with TC (r = 0.927; $p < 0.001$; figure 14), non-HDL-C (r = 0.976; $p < 0.001$; figure 15), oxLDL (r = 0.620; $p < 0.001$; figure 16), and apo B (r = 0.887; $p < 0.001$; figure 17).

Non-HDL-C was positively related with TC (r = 0.935; $p < 0.001$; figure 18), TG (r = 0.304; $p < 0.001$; figure 19), LDL-C (r = 0.976; $p < 0.001$; figure 15), oxLDL (r = 0.672; $p < 0.001$; figure 20), and apo B (r = 0.911; $p < 0.001$; figure 21).

OxLDL showed positive relation to TC (r = 0.584; $p < 0.001$; figure 22), TG (r = 0.332; $p < 0.001$; figure 23), LDL-C (r = 0.620; $p < 0.001$; figure 16), non-HDL-C (r = 0.672; $p < 0.001$; figure 20), and apo B (r = 0.644; $p < 0.001$; figure 24).

Negative correlation was observed between Lp (a) and TG (r = -0.188; p = 0.030; figure 25). The highest correlation was observed between LDL-C and non-HDL-C (r = 0.976; $p < 0.001$; figure 15).

Table 5. Correlation coefficients of lipid parameters in healthy subjects

	TC	TG[a]	HDL-C	LDL-C	Non-HDL-C
TC	1	0.207*	0.119	0.927**	0.935**
TG[a]	0.207*	1	-0.255**	0.107	0.304**
HDL-C	0.119	-0.255**	1	-0.195*	-0.239**
LDL-C	0.927**	0.107	-0.195*	1	0.976**
Non-HDL-C	0.935**	0.304**	-0.239**	0.976**	1
Lp (a)[a]	0.107	-0.188*	0.064	0.118	0.077
OxLDL	0.584**	0.332**	-0.283**	0.620**	0.672**
Apo AI	0.224**	-0.069	0.869**	-0.090	-0.090
Apo B	0.845**	0.290**	-0.240**	0.887**	0.911**

Pearson and [a] Spearman correlations are shown with the level of statistical significance where:
*$p < 0.05$ and **$p < 0.01$.

Table 5. Correlation coefficients of lipid parameters in healthy subjects (continued)

	Lp (a)[a]	OxLDL	Apo AI	Apo B
TC	0.107	0.584**	0.224**	0.845**
TG[a]	-0.188*	0.332**	-0.069	0.290**
HDL-C	0.064	-0.283**	0.869**	-0.240**
LDL-C	0.118	0.620**	-0.090	0.887**
Non-HDL-C	0.077	0.672**	-0.090	0.911**
Lp (a)[a]	1	-0.096	-0.012	0.132
OxLDL	-0.096	1	-0.111	0.644**
Apo AI	-0.012	-0.111	1	-0.110
Apo B	0.132	0.644**	-0.110	1

Pearson and [a] Spearman correlations are shown with the level of statistical significance where:
*$p < 0.05$ and **$p < 0.01$.

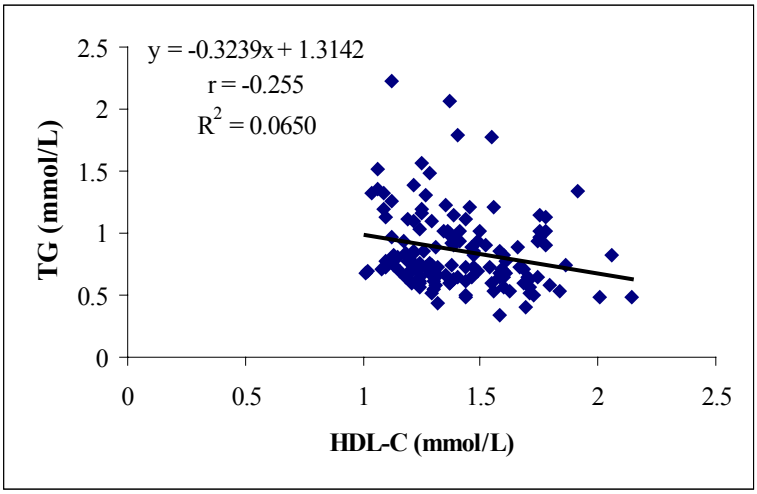

Figure 8. Relationship between HDL-C and TG

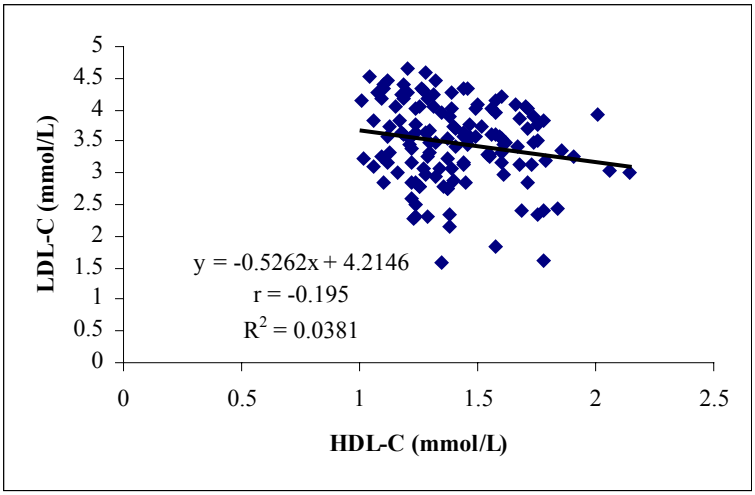

Figure 9. Relationship between HDL-C and LDL-C

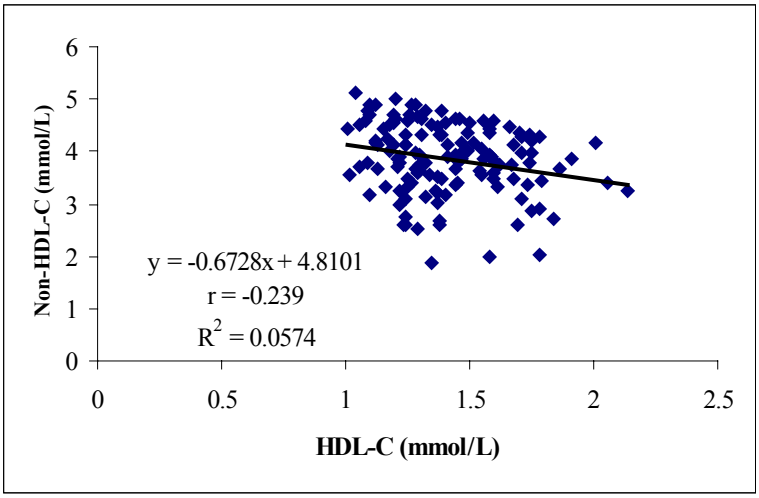

Figure 10. Relationship between HDL-C and non-HDL-C

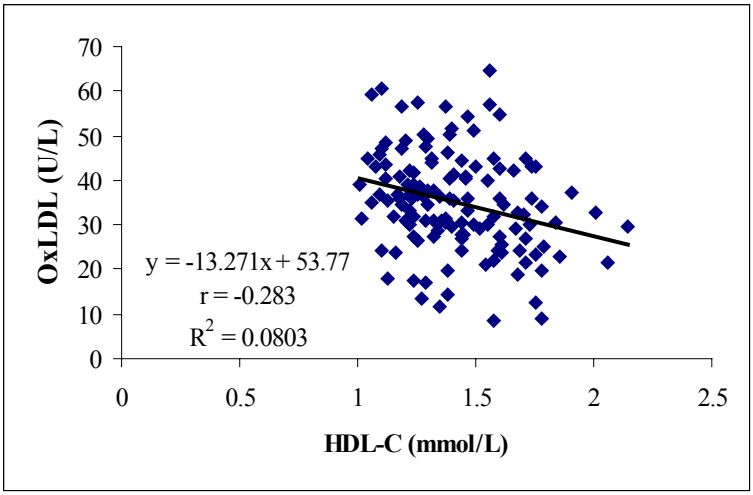

Figure 11. Relationship between HDL-C and oxLDL

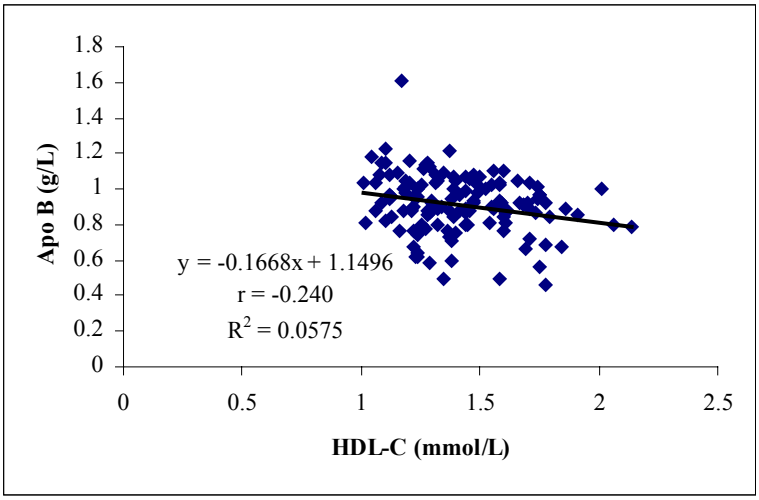

Figure 12. Relationship between HDL-C and apo B

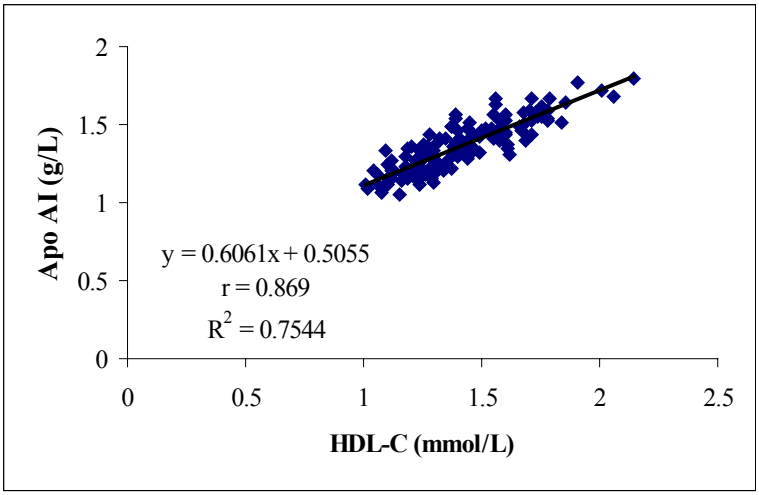

Figure 13. Relationship between HDL-C and apo AI

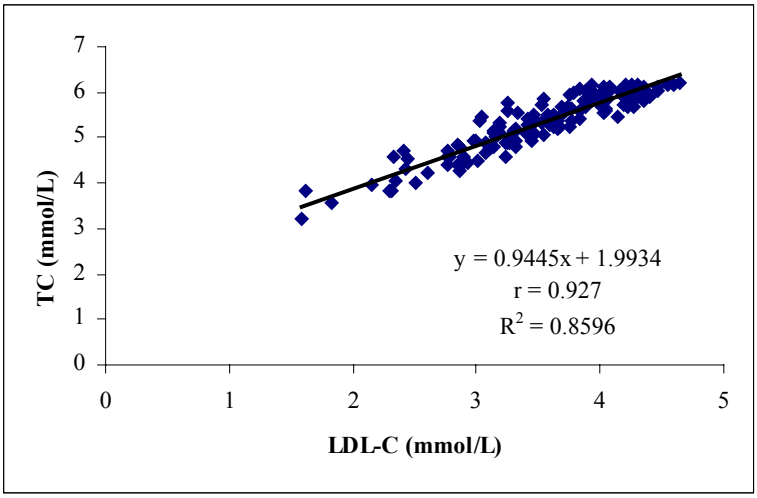

Figure 14. Relationship between LDL-C and TC

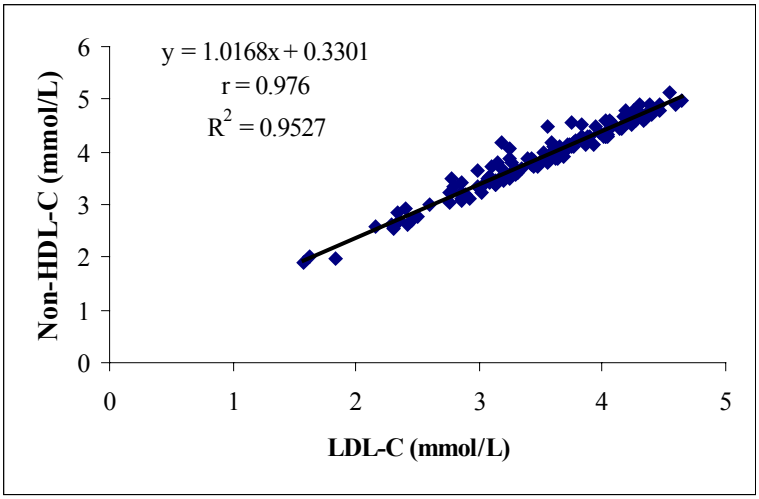

Figure 15. Relationship between LDL-C and non-HDL-C

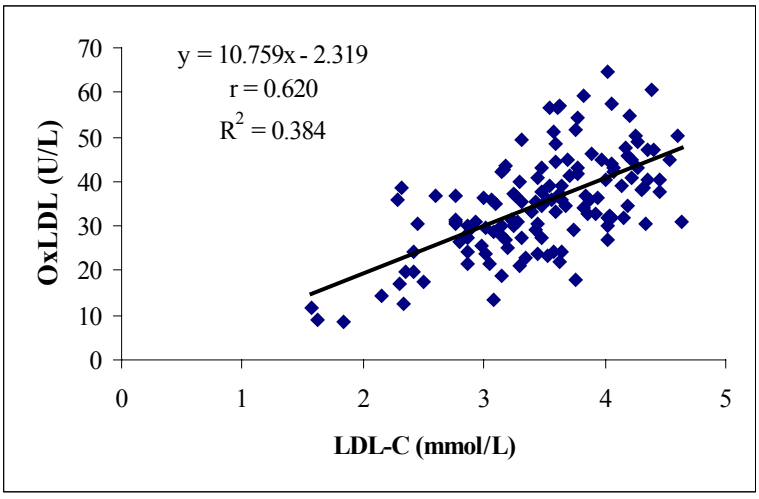

Figure 16. Relationship between LDL-C and oxLDL

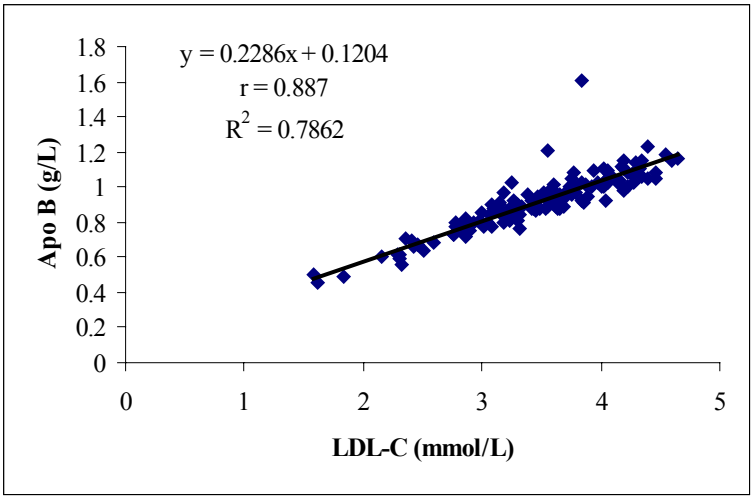

Figure 17. Relationship between LDL-C and apo B

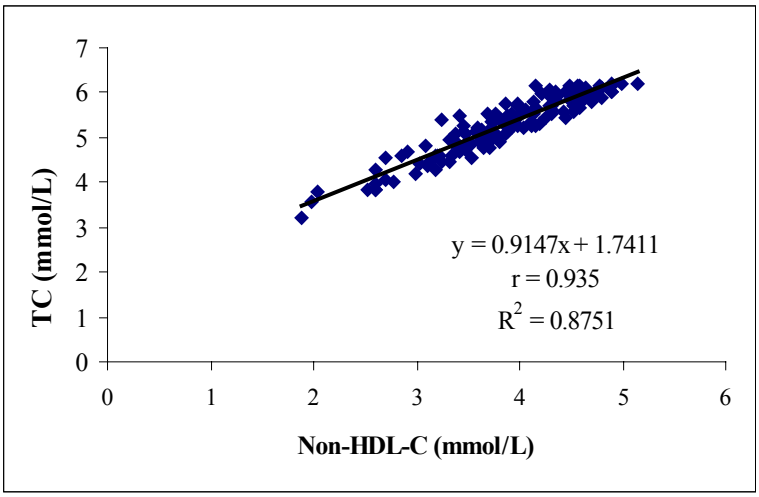

Figure 18. Relationship between non-HDL-C and TC

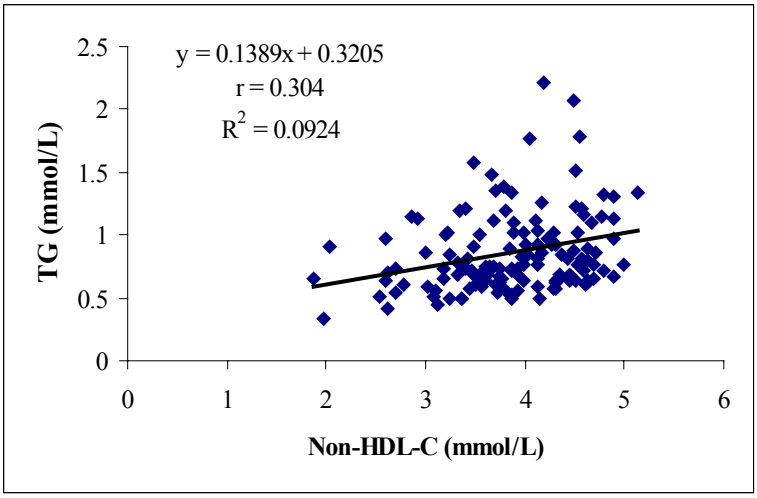

Figure 19. Relationship between non-HDL-C and TG

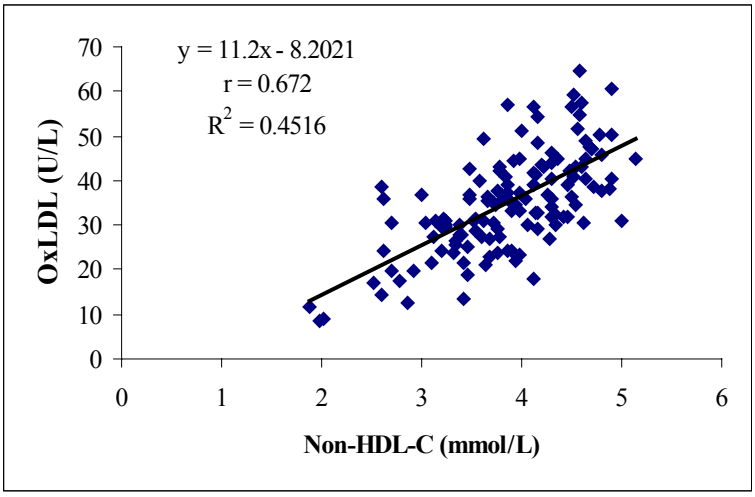

Figure 20. Relationship between non-HDL-C and oxLDL

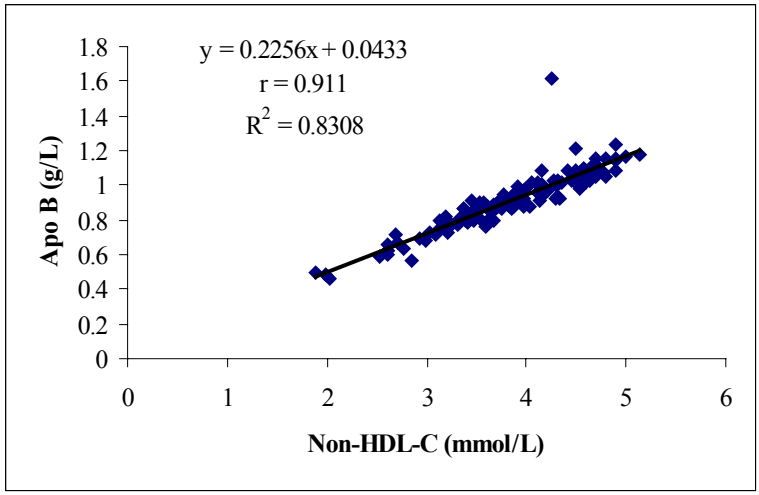

Figure 21. Relationship between non-HDL-C and apo B

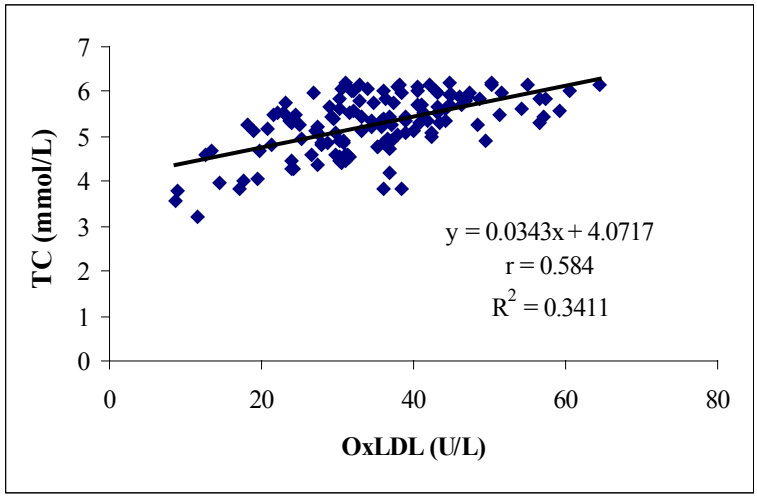

Figure 22. Relationship between oxLDL and TC

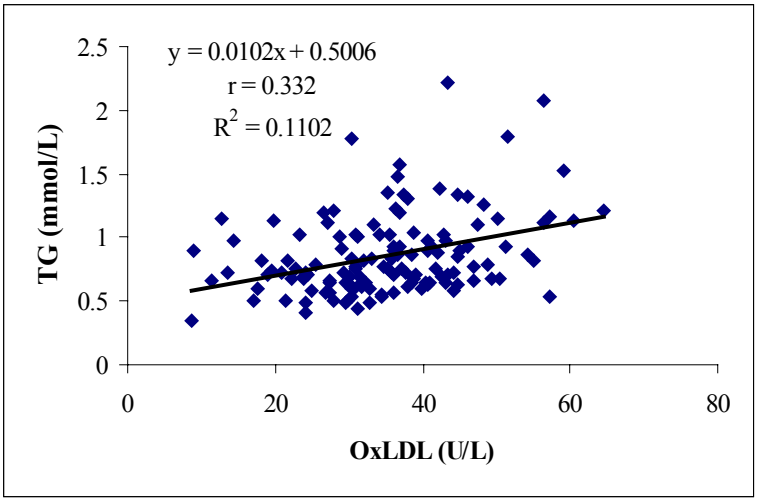

Figure 23. Relationship between oxLDL and TG

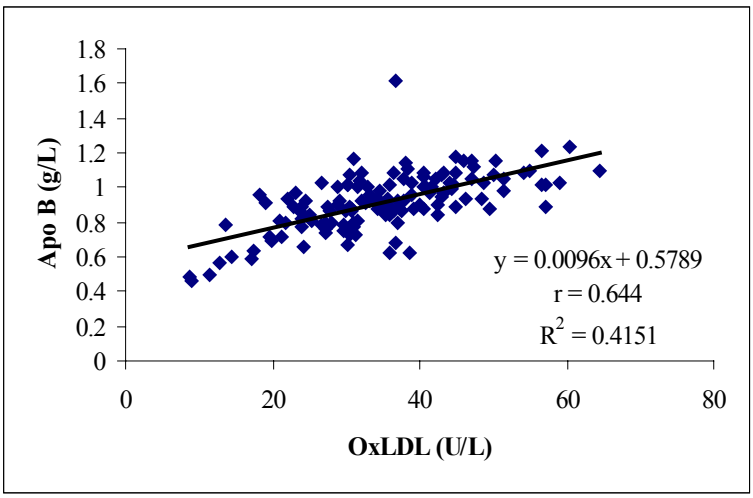

Figure 24. Relationship between oxLDL and apo B

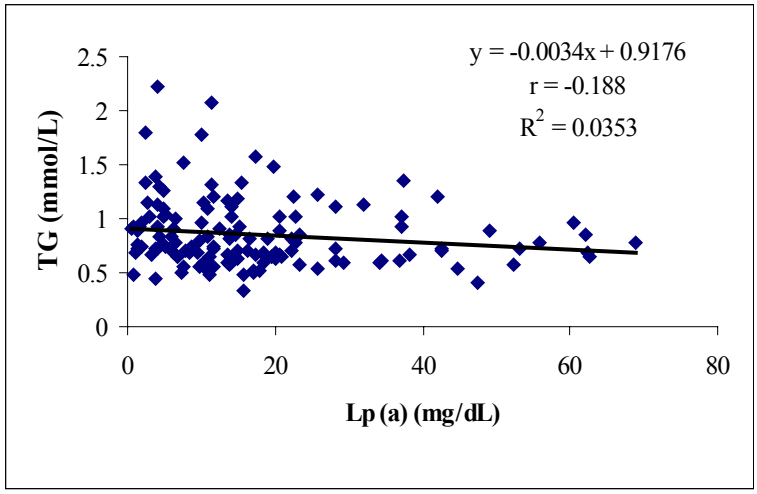

Figure 25. Relationship between Lp (a) and TG

1.5 Correlations between PON1 levels and lipoprotein profiles, WHR, BMI, age, SBP, DBP and FBG

As presented in table 6 and figure 26 to 28, the significantly negative correlations were found between PON1 concentration and WHR (r = -0.232; $p < 0.01$; figure 26), SBP (r = -0.185; $p < 0.05$; figure 27), and DBP (r = -0.196; $p < 0.05$; figure 28). There were no significant correlations between PON1 concentration and any of the lipoprotein parameters. Serum PON1 activity towards paraoxon was positively correlated with HDL-C (r = 0.254; $p < 0.01$; figure 29), apo AI (r = 0.211; $p < 0.05$; figure 30). The serum PON1 activity towards phenylacetate and diazoxon did not show significant correlations with any of the lipoprotein profiles. There were no significant correlations between serum PON1 activity towards the three substrates and age, WHR, BMI, SBP, DBP and FBG as presented in table 6. Amongst PON1 activity towards three substrates, the significantly positive correlation was found between PON1 concentration and PON1 activity towards paraoxon (r = 0.278; $p = 0.001$; figure 31).

Table 6. Correlation coefficients between PON1 levels and lipid parameters, WHR, BMI, age, SBP, DBP and FBG in healthy subjects

Parameters	PON1	PON1 activity towards		
	Concentration	paraoxon[a]	phenylacetate[a]	diazoxon[a]
TC	-0.023	0.071	0.084	-0.068
TG	-0.155	0.081	0.085	-0.001
HDL-C	0.118	0.254**	0.083	-0.091
LDL-C	-0.024	-0.025	-0.001	-0.042
Non-HDL-C	-0.064	-0.036	0.037	-0.027
Lp (a)	-0.012	-0.066	0.000	0.002
OxLDL	0.011	0.019	0.089	-0.015
Apo AI	0.031	0.211*	0.108	-0.008
Apo B	-0.081	-0.001	0.042	-0.055
Age	-0.137	-0.052	0.154	0.035
WHR	-0.232**	-0.048	0.013	0.078
BMI	-0.067	-0.004	0.102	0.094
SBP	-0.185*	0.045	-0.019	-0.104
DBP	-0.196*	0.080	0.014	-0.088
FBG	-0.015	0.152	0.096	-0.018

Pearson and [a] Spearman correlations are shown with the level of statistical significance where: *$p < 0.05$ and **$p < 0.01$.

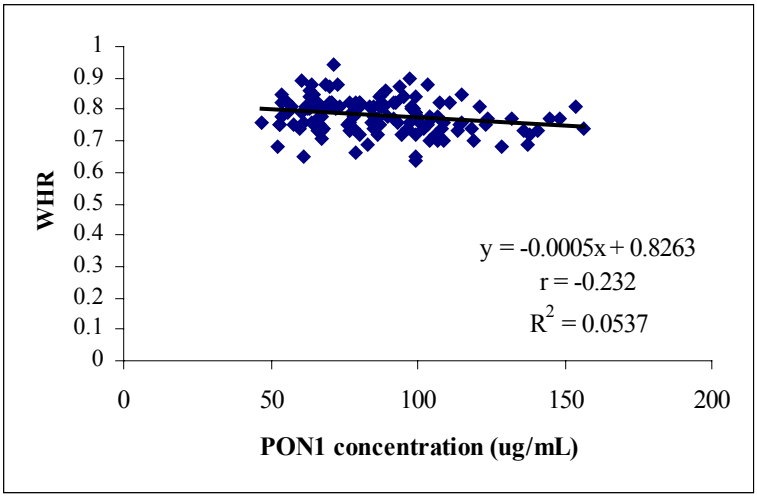

Figure 26. Relationship between PON1 concentration and WHR

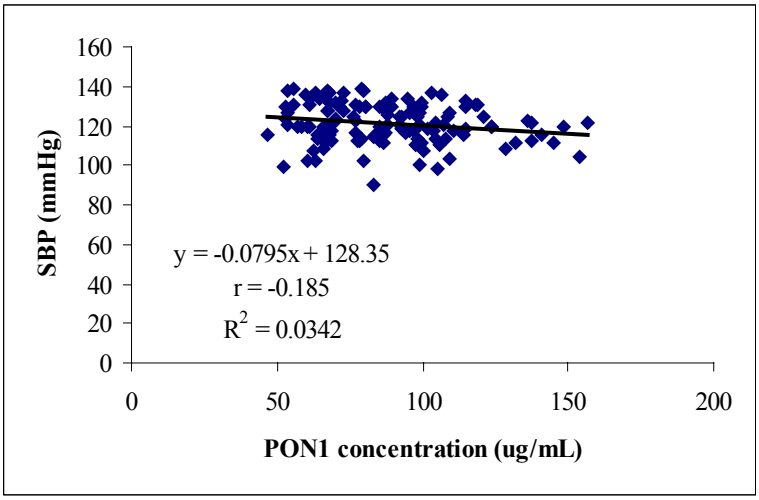

Figure 27. Relationship between PON1 concentration and SBP

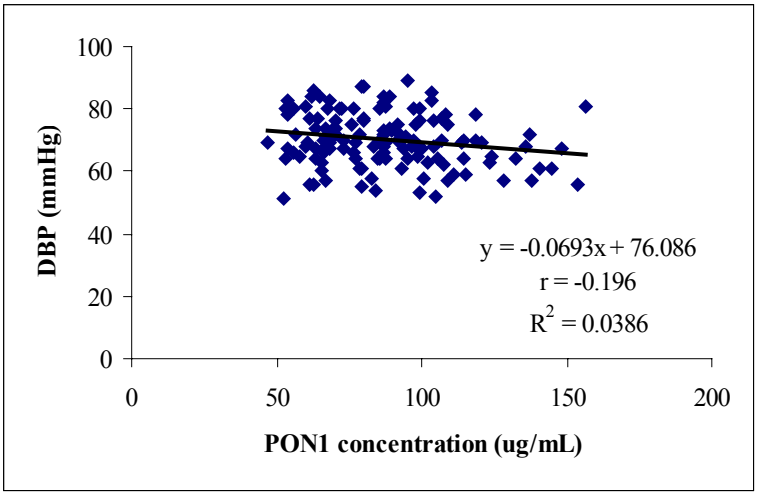

Figure 28. Relationship between PON1 concentration and DBP

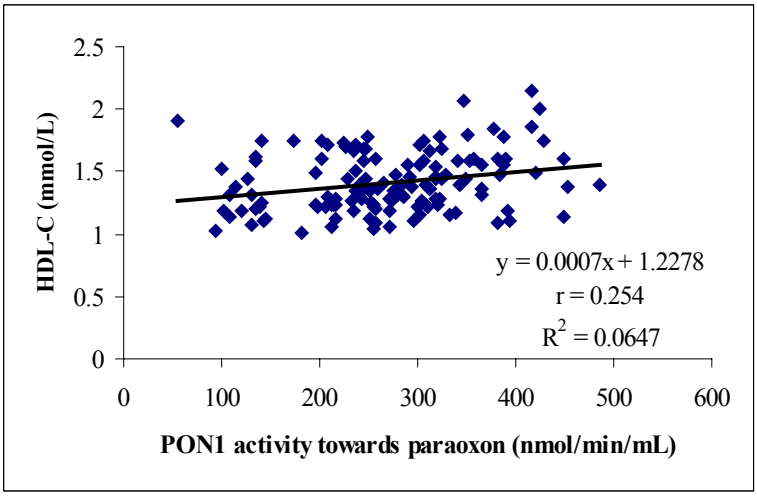

Figure 29. Relationship between PON1 activity towards paraoxon and HDL-C

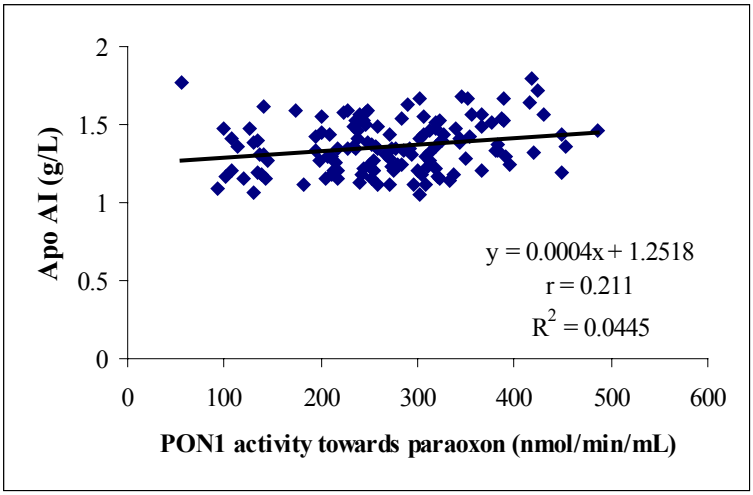

Figure 30. Relationship between PON1 activity towards paraoxon and apo AI

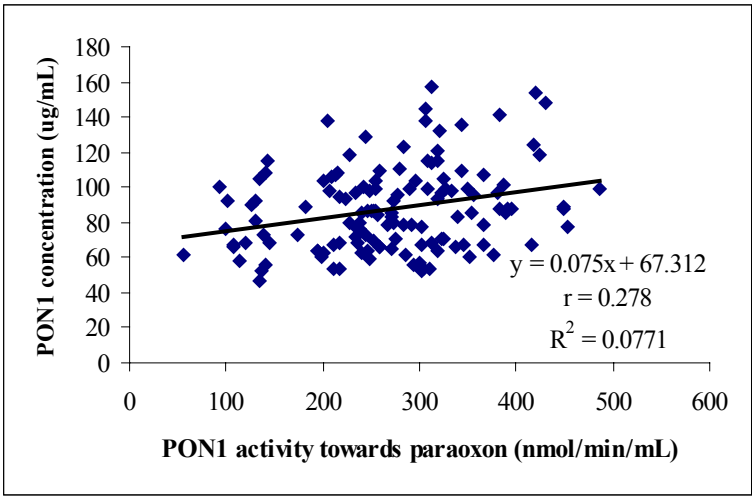

Figure 31. Relationship between PON1 activity towards paraoxon and PON1 concentration

1.6 Genotype and allele frequencies of the *PON1* polymorphisms in healthy subjects

The genotype and allele frequencies of four *PON1* polymorphisms are reported in table 7. Genotype and allele frequencies for the *PON1* L55M, Q192R, T-108C and G-909C polymorphisms were 89.55%LL, 10.45%LM (L=0.95, M=0.05), 50.0%QQ, 41.79%QR, 8.21%RR (Q=0.71, R=0.29), 55.22%TT, 41.05%CT, 3.73%CC (T=0.76, C=0.24), 55.22%CC, 34.33%CG, and 10.45%GG (C=0.72, G=0.28), respectively. The 55LL genotype was the most common genotype found in the population (89.55%), whereas the rarest genotype was the 55MM (0%).

As reported in table 8, a good agreement was found between the observed and expected genotype frequencies at position 55, 192, -108, and -909 according to Hardy-Weinberg equilibrium (p = 0.716, 0.989, 0.392, and 0.265, respectively). These genotype frequencies did not deviate from Hardy-Weinberg equilibrium expectations using the Chi-square test.

The random combination of Q and R alleles at position 192 and L and M alleles at position 55 is expected to give rise to nine possible combinations as shown in table 9. The most common genotypes were 192QQ/55LL (41.79%) and 192QR/55LL (39.55%). However, no 192RR/55MM carriers were observed, suggesting that this genotype combination does not exist or is very rare in this population. Similarly, the most common genotypes from the random combination of T and C alleles at position –108 and C and G alleles at position –909 were -108CT/-909CC (26.87%) and -108TT/-909CC (26.12%). No -108CC/-909GG genotypes were observed in the population.

As presented in table 10, Chi-square test showed significant linkage disequilibrium between Q192R and L55M (p = 0.039), Q192R and T-108C (p = 0.005), and Q192R and G-909C (p = 0.006).

Table 7. Genotype and allele frequencies of *PON1* polymorphisms in healthy subjects

	Polymorphic sites			
	L55M	Q192R	T-108C	G-909C
Genotype frequencies	LL : 89.55 %	QQ : 50.00 %	TT : 55.22 %	CC : 55.22 %
	LM : 10.45 %	QR : 41.79 %	CT : 41.05 %	CG : 34.33 %
	MM : 0 %	RR : 8.21 %	CC : 3.73 %	GG : 10.45 %
Allele frequencies	L : 0.95	Q : 0.71	T : 0.76	C : 0.72
	M : 0.05	R : 0.29	C : 0.24	G : 0.28

Table 8. Assessment of Hardy-Weinberg Equilibrium for the *PON1* polymorphisms in healthy subjects

		Genotype frequencies			
		Observed	Expected	Chi-square value	*p* Value
L55M	LL	120	120.94	0.132	0.716
	LM	14	12.73		
	MM	0	0.33		
Q192R	QQ	67	67.55	0.023	0.989
	QR	56	55.18		
	RR	11	11.27		
T-108C	TT	74	77.40	1.874	0.392
	CT	55	48.88		
	CC	5	7.72		
G-909C	CC	74	69.47	2.655	0.265
	CG	46	54.03		
	GG	14	10.50		

p Value > 0.05 indicates no deviation from Hardy-Weinberg Equilibrium.

Table 9. Distribution of genotypes defined by *PON1* polymorphisms at position 55 vs 192 and –108 vs –909 in healthy subjects

		Q192R		
	QQ	QR	RR	Total
L55M				
LL	56 (41.79%)	53 (39.55%)	11 (8.21%)	120 (89.55%)
LM	11 (8.21%)	3 (2.24%)	0 (0%)	14 (10.45%)
MM	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	67 (50%)	56 (41.79%)	11 (8.21%)	134 (100%)
		G-909C		
	CC	CG	GG	Total
T-108C				
TT	35 (26.12%)	31 (23.13%)	8 (5.97%)	74 (55.22%)
CT	36 (26.87%)	13 (9.70%)	6 (4.48%)	55 (41.05%)
CC	3 (2.24%)	2 (1.49%)	0 (0%)	5 (3.73%)
Total	74 (55.22%)	46 (34.33%)	14 (10.45%)	134 (100%)

Table 10. *p* Values for pairs of *PON1* polymorphisms demonstrating linkage disequilibrium in healthy subjects

	p Value			
	L55M	Q192R	T-108C	G-909C
L55M	.....	0.039	0.487	0.905
Q192R		.....	0.005	0.006
T-108C			.....	0.184
G-909C				.....

Values of *p* are obtained by Chi-square test. *p* Value < 0.05 indicates linkage disequilibrium.

1.7 Influence of the *PON1* polymorphisms on serum PON1 concentration and activity

Table 11 and 12 show serum PON1 concentration and activity towards the three substrates according to four *PON1* polymorphisms. Only the *PON1* L55M polymorphism was associated with significant variation in serum PON1 concentration. The 55LL genotypes had higher PON1 concentration than the 55LM genotypes (89.1 vs 75.2 μg/mL; $p = 0.039$; table 11). There were no significant variations in serum PON1 concentration between genotypes according to the *PON1* Q192R, T-108C, and G-909C polymorphisms ($p = 0.347$, 0.230, and 0.659, respectively; table 11 and 12). Significant variations in serum PON1 activity towards paraoxon as a function of *PON1* Q192R, and G-909C polymorphisms were observed ($p < 0.001$ and $= 0.015$, respectively; table 11 and 12). The 192RR and -909CC genotypes had the highest PON1 activity towards paraoxon (389.9 and 290.7 nmol/min/mL), whereas the 192QQ and -909GG genotypes had the lowest activity (242.5 and 189.9 nmol/min/mL) and the 192QR and -909CG heterozygotes having intermediate activity (303.7 and 248.2 nmol/min/mL). None of *PON1* polymorphisms significantly affected the variation in serum PON1 activity towards phenylacetate. The *PON1* Q192R and G-909C polymorphisms influenced diazoxon hydrolysis by PON1 ($p < 0.001$; table 11 and 12). In contrast to the influences on serum PON1 activity towards paraoxon, the 192RR and –909CC genotypes had the lowest serum PON1 activity towards diazoxon (7.5 and 7.7 μmol/min/mL), whereas the 192QQ and -909GG genotypes had the highest activity (11.7 and 13.9 μmol/min/mL) and the 192QR and -909CG heterozygotes had intermediate activity (7.9 and 10.9 μmol/min/mL). Interestingly, the *PON1* T-108C polymorphism did not significantly affect the variation in serum PON1 activity towards any substrate, although the -108CT genotypes had the highest serum PON1 activity towards paraoxon (299.3 nmol/min/mL) and phenylacetate (76.5 μ mol/min/mL), and had the lowest activity towards diazoxon (8.6 μmol/min/mL), these differences did not achieve statistical significance ($p = 0.280$, 0.063 and 0.106, respectively; table 12).

Table 11. PON1 concentration and activity towards the three substrates according to the *PON1* L55M and Q192R polymorphisms in healthy subjects

PON1 levels	L55M	Q192R
Concentration (μg/mL)	LL: 89.1 ± 24.2	QQ: 85.2 ± 23.8
	LM: 75.2 ± 18.1	QR: 88.9 ± 23.4
		RR: 95.7 ± 27.2
	p Value = 0.039	*p* Value = 0.347
Activity towards paraoxon [a] (nmol/min/mL)	LL: 272.0 (55.5-486.2)	QQ: 242.5 (55.5-420.2)
	LM: 237.3 (100.2-351.6)	QR: 303.7 (173.7-423.9)
		RR: 389.9 (251.9-486.2)
	p Value = 0.056	*p* Value < 0.001
Activity towards phenylacetate [a] (μmol/min/mL)	LL: 75.4 (26.9-103.6)	QQ: 74.1 (42.1-103.6)
	LM: 71.2 (42.1-88.5)	QR: 73.9 (26.9-100.3)
		RR: 77.3 (44.3-98.8)
	p Value = 0.132	*p* Value = 0.400
Activity towards diazoxon [a] (μmol/min/mL)	LL: 9.8 (3.9-21.4)	QQ: 11.7 (1.2-21.4)
	LM: 8.5 (1.1-19.1)	QR: 7.9 (4.4-15.0)
		RR: 7.5 (3.9-9.1)
	p Value = 0.613	*p* Value < 0.001

Values are shown as mean ± SD. [a]Values are median (range).

Table 12. PON1 concentration and activity towards the three substrates according to the *PON1* T-108C and G-909C polymorphisms in healthy subjects

PON1 levels	T-108C	G-909C
Concentration (μg/mL)	TT: 84.4 ± 21.5 CT: 91.5 ± 26.2 CC: 92.7 ± 30.5 *p* Value = 0.230	CC: 88.6 ± 24.2 CG: 87.6 ± 23.3 GG: 82.2 ± 25.6 *p* Value = 0.659
Activity towards paraoxon[a] (nmol/min/mL)	TT: 268.7 (108.1-453.1) CT: 299.3 (93.7-486.2) CC: 216.7 (55.5-344.5) *p* Value = 0.280	CC: 290.7 (100.2-486.2) CG: 248.2 (55.5-449.6) GG: 189.9 (93.7-453.1) *p* Value = 0.015
Activity towards phenylacetate[a] (μmol/min/mL)	TT: 73.8 (26.9-103.6) CT: 76.5 (44.3-100.3) CC: 56.8 (43.9-76.4) *p* Value = 0.063	CC: 73.9 (26.9-100.3) CG: 73.5 (43.7-103.6) GG: 82.0 (51.8-97.7) *p* Value = 0.123
Activity towards diazoxon[a] (μmol/min/mL)	TT: 10.9 (4.2-21.4) CT: 8.6 (1.2-19.1) CC: 11.1 (5.9-17.8) *p* Value = 0.106	CC: 7.7 (1.2-19.1) CG: 10.9 (4.9-21.4) GG: 13.9 (7.1-21.3) *p* Value < 0.001

Values are shown as mean ± SD. [a]Values are median (range).

1.8 Influence of the *PON1* polymorphisms on lipoprotein profiles

Table 13 and 14 show lipid parameters according to four *PON1* polymorphisms. The *PON1* Q192R and G-909C polymorphisms significantly influenced the variation of some lipid parameters. There were significant differences in TG ($p = 0.017$) and HDL-C ($p = 0.028$) levels between genotypes classified by the *PON1* Q192R polymorphism (table 13). The apo AI level increased in order of genotype from –909GG to -909CG to –909CC ($p = 0.042$; table 14).

Table 13. Lipid parameters according to the *PON1* L55M and Q192R polymorphisms in healthy subjects

Parameters	L55M	Q192R
TC	LL: 5.27 ± 0.66	QQ: 5.18 ± 0.73
(mmol/L)	LM: 5.31 ± 0.57	QR: 5.31 ± 0.56
		RR: 5.60 ± 0.41
	p Value = 0.843	*p* Value = 0.117
TG [a]	LL: 0.76 (0.34-2.22)	QQ: 0.86 (0.34-2.22)
(mmol/L)	LM: 0.82 (0.58-1.57)	QR: 0.73 (0.41-1.77)
		RR: 0.75 (0.56-1.79)
	p Value = 0.371	*p* Value = 0.017
HDL-C	LL: 1.42 ± 0.24	QQ: 1.36 ± 0.22
(mmol/L)	LM: 1.39 ± 0.21	QR: 1.47 ± 0.25
		RR: 1.44 ± 0.23
	p Value = 0.671	*p* Value = 0.028
LDL-C	LL: 3.47 ± 0.65	QQ: 3.40 ± 0.74
(mmol/L)	LM: 3.51 ± 0.55	QR: 3.49 ± 0.52
		RR: 3.77 ± 0.40
	p Value = 0.803	*p* Value = 0.196
Non-HDL-C	LL: 3.85 ± 0.68	QQ: 3.82 ± 0.79
(mmol/L)	LM: 3.91 ± 0.53	QR: 3.84 ± 0.52
		RR: 4.16 ± 0.45
	p Value = 0.730	*p* Value = 0.299

Values are shown as mean ± SD. [a]Values are shown by median (range)

Table 13. Lipid parameters according to the *PON1* L55M and Q192R polymorphisms in healthy subjects (continued)

Parameters	L55M	Q192R
Lp (a)[a]	LL: 13.70 (0.65-62.56)	QQ: 13.93 (0.65-68.77)
(mg/dL)	LM: 13.89 (1.04-68.77)	QR: 14.34 (0.70-62.56)
		RR: 8.48 (1.64-62.35)
	p Value = 0.760	*p* Value = 0.283
OxLDL	LL: 35.18 ± 11.32	QQ: 36.01 ± 13.24
(U/L)	LM: 33.73 ± 9.07	QR: 33.32 ± 8.32
		RR: 37.79 ± 8.12
	p Value = 0.643	*p* Value = 0.283
Apo AI	LL: 1.36 ± 0.17	QQ: 1.33 ± 0.16
(g/L)	LM: 1.38 ± 0.16	QR: 1.39 ± 0.16
		RR: 1.36 ± 0.17
	p Value = 0.734	*p* Value = 0.073
Apo B	LL: 0.91 ± 0.17	QQ: 0.90 ± 0.19
(g/L)	LM: 0.92 ± 0.12	QR: 0.92 ± 0.14
		RR: 0.97 ± 0.09
	p Value = 0.956	*p* Value = 0.438

Values are shown as mean ± SD. [a]Values are shown by median (range).

Table 14. Lipid parameters according to the *PON1* T-108C and G-909C polymorphisms in healthy subjects

Parameters	T-108C	G-909C
TC	TT: 5.30 ± 0.67	CC: 5.24 ± 0.66
(mmol/L)	CT: 5.25 ± 0.60	CG: 5.31 ± 0.64
	CC: 5.15 ± 1.00	GG: 5.34 ± 0.66
	p Value = 0.841	*p* Value = 0.761
TG [a]	TT: 0.78 (0.41-2.07)	CC: 0.76 (0.34-2.07)
(mmol/L)	CT: 0.73 (0.44-2.22)	CG: 0.85 (0.44-1.48)
	CC: 0.78 (0.34-1.34)	GG: 0.76 (0.54-2.22)
	p Value = 0.349	*p* Value = 0.418
HDL-C	TT: 1.40 ± 0.21	CC: 1.44 ± 0.24
(mmol/L)	CT: 1.42 ± 0.27	CG: 1.40 ± 0.23
	CC: 1.56 ± 0.24	GG: 1.28 ± 0.22
	p Value = 0.331	*p* Value = 0.065
LDL-C	TT: 3.50 ± 0.68	CC: 3.42 ± 0.65
(mmol/L)	CT: 3.46 ± 0.55	CG: 3.51 ± 0.59
	CC: 3.22 ± 0.99	GG: 3.65 ± 0.75
	p Value = 0.613	*p* Value = 0.401
Non-HDL-C	TT: 3.90 ± 0.69	CC: 3.79 ± 0.67
(mmol/L)	CT: 3.83 ± 0.59	CG: 3.91 ± 0.62
	CC: 3.59 ± 1.06	GG: 4.05 ± 0.75
	p \Value = 0.556	*p* Value = 0.338

Values are shown as mean ± SD. [a]Values are shown by median (range)

Table 14. Lipid parameters according to the *PON1* T-108C and G-909C polymorphisms in healthy subjects (continued)

Parameters	T-108C	G-909C
Lp (a)[a]	TT: 14.47 (0.65-62.56)	CC: 13.77 (0.65-62.08)
(mg/dL)	CT: 11.60 (0.70-68.77)	CG: 14.08 (1.04-62.56)
	CC: 15.61 (2.39-22.77)	GG: 12.66 (1.80-68.77)
	p Value = 0.724	*p* Value = 0.992
OxLDL	TT: 35.76 ± 12.61	CC: 34.41 ± 11.03
(U/L)	CT: 34.37 ± 8.20	CG: 36.05 ± 11.04
	CC: 31.47 ± 15.57	GG: 34.96 ± 12.07
	p Value = 0.600	*p* Value = 0.734
Apo AI	TT: 1.36 ± 0.16	CC: 1.38 ± 0.16
(g/L)	CT: 1.35 ± 0.17	CG: 1.37 ± 0.17
	CC: 1.46 ± 0.18	GG: 1.26 ± 0.12
	p Value = 0.401	*p* Value = 0.042
Apo B	TT: 0.93 ± 0.18	CC: 0.90 ± 0.16
(g/L)	CT: 0.90 ± 0.13	CG: 0.93 ± 0.17
	CC: 0.84 ± 0.24	GG: 0.94 ± 0.17
	p Value = 0.361	*p* Value = 0.546

Values are shown as mean ± SD. [a]Values are shown by median (range).

1.9 Parameters influencing serum PON1 concentration and activity

Parameters influencing serum PON1 concentration and activity towards the three substrates were analyzed using stepwise multiple regression analysis as shown in table 15. Independent parameters, including all four *PON1* polymorphisms, PON1 concentration, HDL-C and apo AI concentrations were used as predictors of serum PON1 concentration and activity in stepwise multiple regression analysis. Only the *PON1* L55M polymorphism was identified as a significant contributor to PON1 concentration, accounting for 3.2% of the variation in serum PON1 concentration (p = 0.039; table 15). The *PON1* Q192R polymorphism and PON1 concentration were significant contributors to serum PON1 activity towards paraoxon, accounting for 26.0% of the variation in this activity for Q192R ($p < 0.001$; table 15) and 30.6% for Q192R together with PON1 concentration (p = 0.004; table 15). However, all independent parameters were not identified as contributors for PON1 activity towards phenylacetate (data not shown). Finally, significant contributions were also observed for the *PON1* G-909C and Q192R polymorphisms to variation in serum PON1 activity towards diazoxon, accounting for 21.7% of the variation in this activity for G-909C ($p < 0.001$; table 15), 30.1% for G-909C together with Q192R ($p < 0.001$; table 15).

Table 15. Stepwise multiple regression analysis of parameters influencing serum PON1 concentration and activity in healthy subjects

		Variation (%)	*p* Value
PON1 concentration			
Model A parameters[1]:	L55M	3.2	0.039
	T-108C		0.129
	HDL-C		0.193
	Q192R		0.291
	G-909C		0.385
	Apo AI		0.672

[1]Predictors in the Model A: (constant), L55M

Table 15. Stepwise multiple regression analysis of parameters influencing serum PON1 concentration and activity in healthy subjects (continued)

		Variation (%)	*p* Value
PON1 activity towards paraoxon			
Model A parameters[2]:	Q192R	26.0	<0.001
	PON1 concentration		0.004
	HDL-C		0.035
	G-909C		0.052
	Apo AI		0.056
	T-108C		0.311
	L55M		0.353
Model B parameters[3]:	Q192R and PON1 concentration	30.6	0.004
	Apo AI		0.054
	HDL-C		0.059
	G-909C		0.059
	T-108C		0.147
	L55M		0.621
PON1 activity towards diazoxon			
Model A parameters[4]:	G-909C	21.7	<0.001
	Q192R		0.000
	PON1 concentration		0.075
	T-108C		0.432
	Apo AI		0.526
	L55M		0.532
	HDL-C		0.723
Model B parameters[5]:	G-909C and Q192R	30.1	<0.001
	L55M		0.123
	PON1 concentration		0.147
	Apo AI		0.276
	T-108C		0.587
	HDL-C		0.818

[2]Predictors in the Model A: (constant), Q192R

[3]Predictors in the Model B: (constant), Q192R, PON1 concentration

[4]Predictors in the Model A: (constant), G-909C

[5]Predictors in the Model B: (constant), G-909C, Q192R

2. Part 2: The study of effect of fenofibrate therapy on PON1 status in patients with low HDL-C levels

A total of 61 male dyslipidemic patients with low HDL-C levels were recruited into the study after dietary run-in period. Fifty-eight of 61 patients (95.1%) completed the study. The withdrawal rate in this study was 4.9% (3/61). After 6 weeks of therapy, two patients withdrew due to adverse events (rash) and one patient discontinued treatment due to loss of follow up.

2.1 Demographic characteristics of patients with low HDL-C levels

Demographic details of patients with low HDL-C levels are presented in table 16. Mean age, waist circumference, WHR, BW, BMI, SBP, DBP, and FBG were 44.0 years, 92.16 cm, 0.90, 74.58 kg, 26.3 kg/m^2, 126.3 mmHg and 73.7 mmHg, 5.17 mmol/L, respectively. Thirty-four of 61 patients (55.74%) were smokers.

Table 16. Demographic details of patients with low HDL-C levels

	Low HDL-C patients (n = 61)
Age (years)	44.0 ± 6.4
Waist circumference (cm)	92.16 ± 7.12
WHR	0.90 ± 0.04
BW (kg)	74.58 ± 9.99
BMI (kg/m^2)	26.3 ± 3.0
SBP (mmHg)	126.3 ± 12.6
DBP (mmHg)	73.7 ± 9.8
FBG (mmol/L)	5.17 ± 0.62

Values are shown as mean ± SD.

2.2 Baseline lipid status of patients with low HDL-C levels

The baseline lipid parameters of the patients are summarized in table 17. The average values of TC, TG, HDL-C, LDL-C, and non-HDL-C were 5.91, 2.85, 0.91, 3.70, and 5.01 mmol/L, respectively. The mean values of oxLDL, apo AI and apo B concentrations were 64.63 U/L, 1.11 g/L and 1.15 g/L, respectively.

Table 17. Lipid parameters and hs-CRP at baseline, 6 and 12 weeks after microcoated fenofibrate treatment in patients with low HDL-C levels

	Baseline	6 weeks	12 weeks
TC (mmol/L)	5.91 ± 0.87	5.51 ± 0.97**	5.50 ± 0.99**
% Change ▲		-6.41	-6.41
TG (mmol/L)[a]	2.85 (1.45-4.50)	1.72 (0.73-3.72)**	1.46 (0.8-3.80)**
% Change▲		-34.63	-40.58
HDL-C (mmol/L)	0.91 ± 0.08	1.04 ± 0.19*	1.05 ± 0.21*
% Change▲		+14.68	+17.28
LDL-C (mmol/L)	3.70 ± 0.85	3.67 ± 0.78	3.71 ± 0.86
% Change▲		+1.88	+3.35
Non-HDL-C (mmol/L)	5.01 ± 0.84	4.47 ± 0.90**	4.44 ± 0.96**
% Change▲		-10.09	-10.58
OxLDL (U/L)	64.63 ± 29.64	53.38 ± 16.71**	50.46 ± 14.96**
% Change▲		-13.35	-16.11
Apo AI (g/L)	1.11 ± 0.12	1.19 ± 0.17*	1.19 ± 0.23*
% Change▲		+6.89	+7.25
Apo B (g/L)	1.15 ± 0.19	1.05 ± 0.19**	1.06 ± 0.21**
% Change▲		-8.15	-7.28
hs-CRP (mg/L)	1.95 ± 2.04	1.75 ± 2.53	1.69 ± 1.65
% Change▲		+10.40	+17.87

Values are shown as mean ± SD. [a]Values are shown as median (range).

▲% Change is the mean of % change from individual patient.

Significant differences from baseline are given: * $p < 0.01$ and ** $p < 0.001$.

2.3 Effects of microcoated fenofibrate on lipid parameters in patients with low HDL-C levels

As shown in table 17 and figure 32, microcoated fenofibrate significantly decreased TC, TG, non-HDL-C, oxLDL and apo B levels by -6.41%, -34.63%, -10.09%, -13.35%, and -8.15% after 6 weeks of treatment, respectively. Meanwhile, HDL-C and apo AI levels were significantly increased by +14.68% and +6.89% after 6 weeks of treatment, respectively. Similarly, the mean of TC, TG, non-HDL-C, oxLDL, and apo B levels were significantly reduced by -6.41%, -40.58%, -10.58%, and -7.28% after 12 weeks of treatment, respectively. Whereas, HDL-C and apo AI levels were significantly increased by +17.28% and +7.25% after 12 weeks of treatment, respectively. However, there was no significant difference in the mean of LDL-C at week 6 and 12 after treatment when compared with those at baseline. The difference in change of lipid parameters between 6 and 12 weeks after treatment was not statistically significant ($p > 0.05$).

The change of HDL-C negatively correlated with the change of TG ($r = -0.453$; $p < 0.01$; figure 33), non-HDL-C ($r = -0.268$; $p < 0.05$; figure 34) and oxLDL ($r = -0.303$; $p < 0.05$; figure 35). The significantly negative correlations were also detected between the change of apo AI and the change of TG ($r = -0.326$; $p < 0.05$; figure 36) and non-HDL-C ($r = -0.323$; $p < 0.05$; figure 37). In addition, there was a non-significantly negative correlation between the change of apo AI and the change of oxLDL ($r = -0.248$; $p > 0.05$; figure 38).

The percentage of patients with baseline HDL-C levels of less than 40 mg/dL (1.03 mmol/L) achieving HDL-C treatment goal, HDL-C levels ≥ 40 mg/dL, after 6 and 12 weeks of microcoated fenofibrate therapy were 47.5% (29/61) and 55.2% (32/58), respectively. None of patients achieving HDL-C ≥ 60 mg/dL (1.55 mmol/L) was observed after therapy.

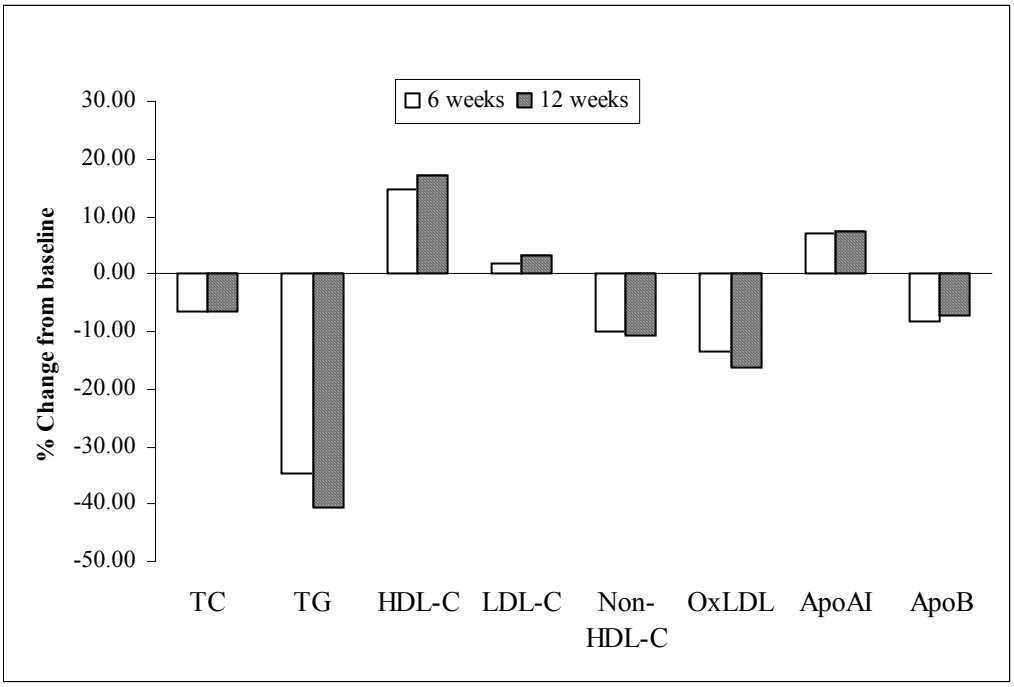

Figure 32. Percentage change from baseline of lipid parameters after 6 and 12 weeks of microcoated fenofibrate treatment

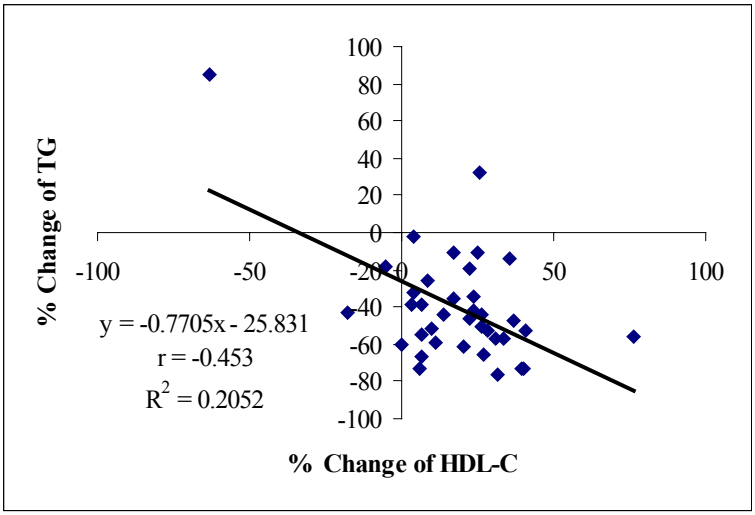

Figure 33. Relationship between % change of HDL-C and % change of TG

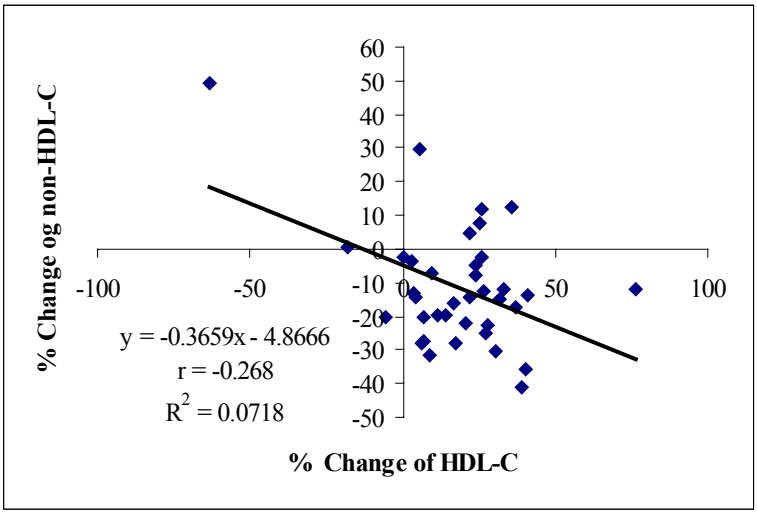

Figure 34. Relationship between % change of HDL-C and % change of non-HDL-C

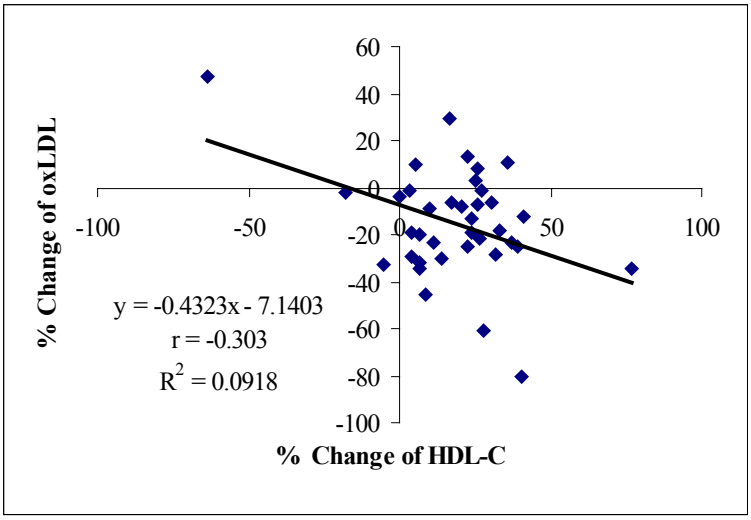

Figure 35. Relationship between % change of HDL-C and % change of oxLDL

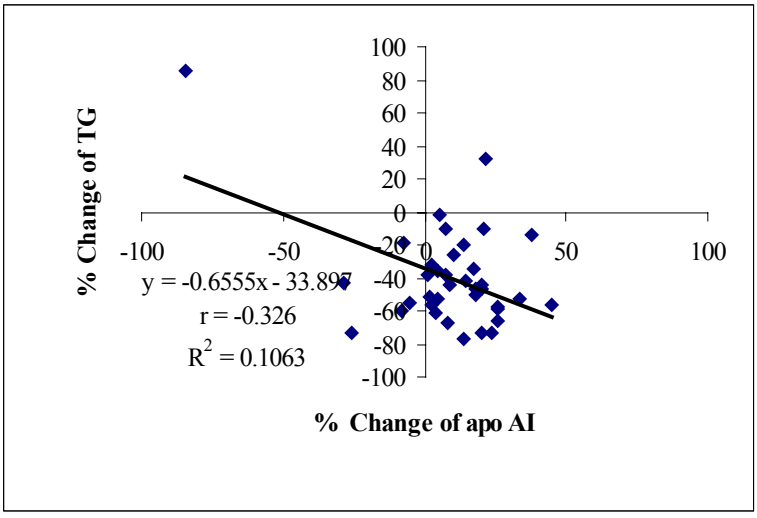

Figure 36. Relationship between % change of apo AI and % change of TG

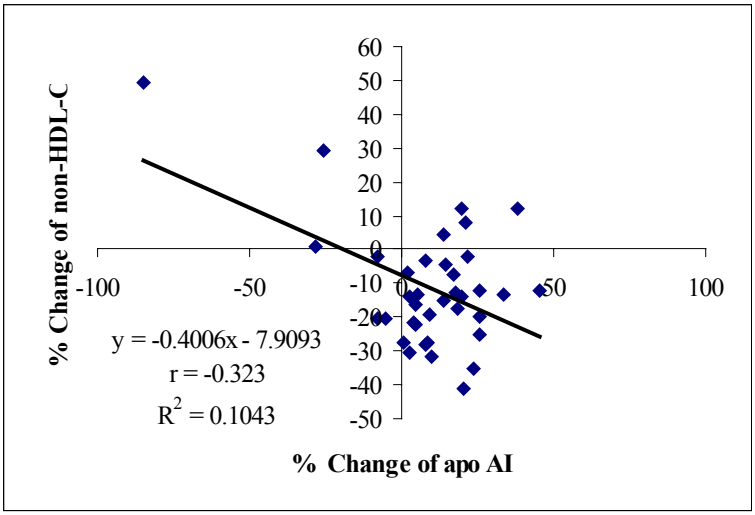

Figure 37. Relationship between % change of apo AI and % change of non-HDL-C

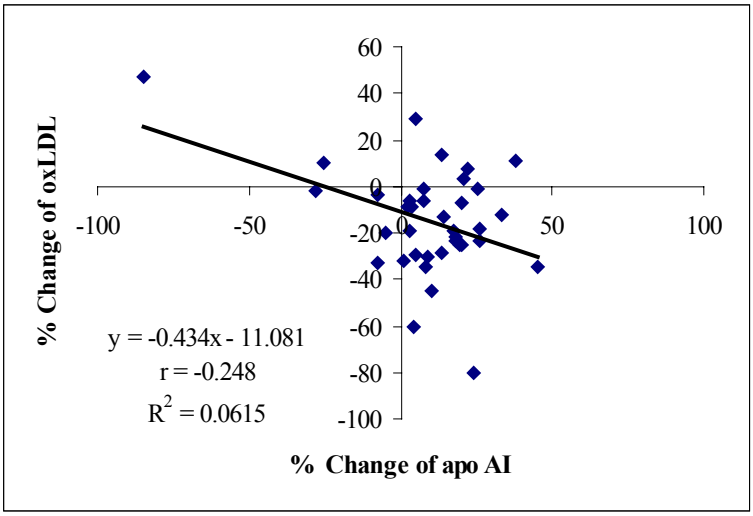

Figure 38. Relationship between % change of apo AI and % change of oxLDL

2.4 Baseline hs-CRP of patients with low HDL-C levels

The mean value of hs-CRP was 1.95 mg/L as shown in table 17. Baseline hs-CRP was positively correlated with waist circumference (r = 0.286; p = 0.026; figure 39) and BMI (r = 0.280; p = 0.029; figure 40).

The patients were stratified into 3 subgroups according to their baseline hs-CRP levels as demonstrated in table 18. The first, second and third groups included 41% (25/61), 37.7% (23/61)and 21.3% (13/61) of the patients with baseline hs-CRP levels of < 1, 1-3 and > 3 mg/L, respectively. There was no patient with baseline hs-CRP levels of > 10 mg/L reflecting an active infection or inflammation.

Additionally, the patients were also further classified by smoking as shown in table 18. It was observed that baseline hs-CRP levels did not differ significantly between nonsmokers and smokers (1.84 vs 2.05 mg/L; p = 0.697; table 18).

2.5 Effect of microcoated fenofibrate on hs-CRP in patients with low HDL-C levels

After 6 and 12 weeks of microcoated fenofibrate treatment, there was no significant difference in the mean of hs-CRP when compared with those at baseline (table 17). Nonetheless, it was found that fenofibrate therapy led to the reduction of hs-CRP from baseline in 65.6% (40/61) and 48.3% (28/58) of patients after 6 and 12 weeks, respectively (figure 41).

Analysis according to baseline hs-CRP levels demonstrated that in the patients with baseline hs-CRP levels > 3 mg/L, microcoated fenofibrate significantly reduced the mean hs-CRP from 5.23 to 3.31 mg/L after 12 weeks of treatment (p = 0.032; table 18). Furthermore, it was observed that 53.9% (7/13) of patients with baseline hs-CRP levels of > 3 mg/L achieved hs-CRP level of < 3 mg/L after fenofibrate treatment. In contrast, there was no significant difference in the mean hs-CRP after treatment in the other two groups of the patients with baseline hs-CRP levels of < 1 and 1-3 mg/L (table 18).

In both nonsmokers and smokers, the mean values of hs-CRP after microcoated fenofibrate treatment did not differ significantly when compared with those at baseline as presented in table 18.

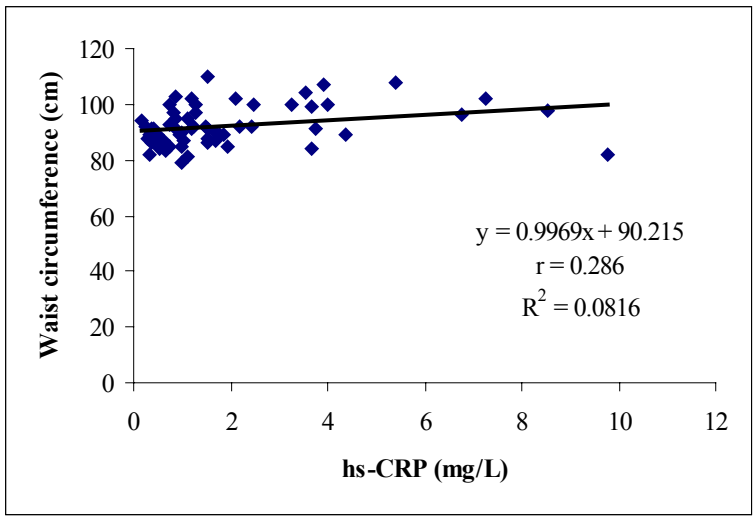

Figure 39. Relationship between hs-CRP and waist circumference

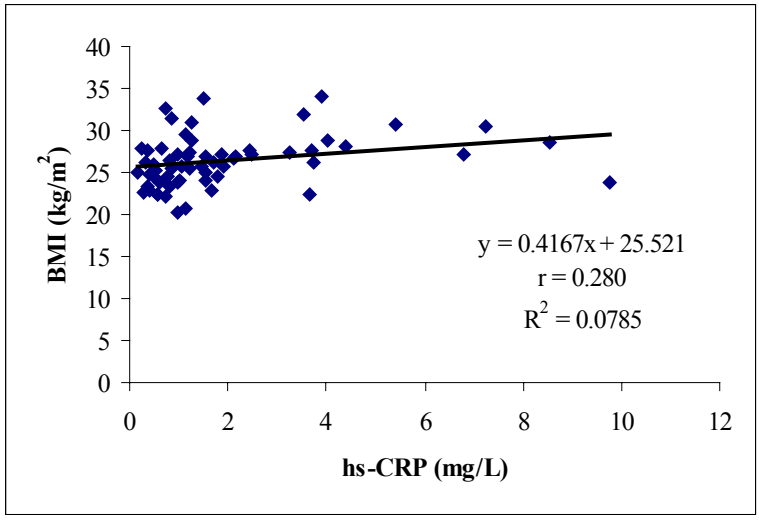

Figure 40. Relationship between hs-CRP and BMI

Table 18. Effect of microcoated fenofibrate treatment on serum hs-CRP levels classified by baseline hs-CRP levels and smoking habit

	Serum hs-CRP levels (mg/L)		
	Baseline	6 weeks	12 weeks
Subgroup			
hs-CRP < 1 mg/L (n=25)	0.60 ± 0.24	0.79 ± 0.91	0.83 ± 0.60
hs-CRP 1-3 mg/L (n=23)	1.57 ± 0.43	1.31 ± 0.83	1.67 ± 1.26
hs-CRP > 3 mg/L (n=13)	5.23 ± 2.15	4.37 ± 4.41	3.31 ± 2.30*
Subgroup			
Nonsmokers (n=27)	1.84 ± 1.64	1.97 ± 3.47	1.67 ± 1.23
Smokers (n=34)	2.05 ± 2.33	1.57 ± 1.46	1.71 ± 1.95

Values are shown as mean ± SD.

Significant differences from baseline are given: *$p < 0.05$.

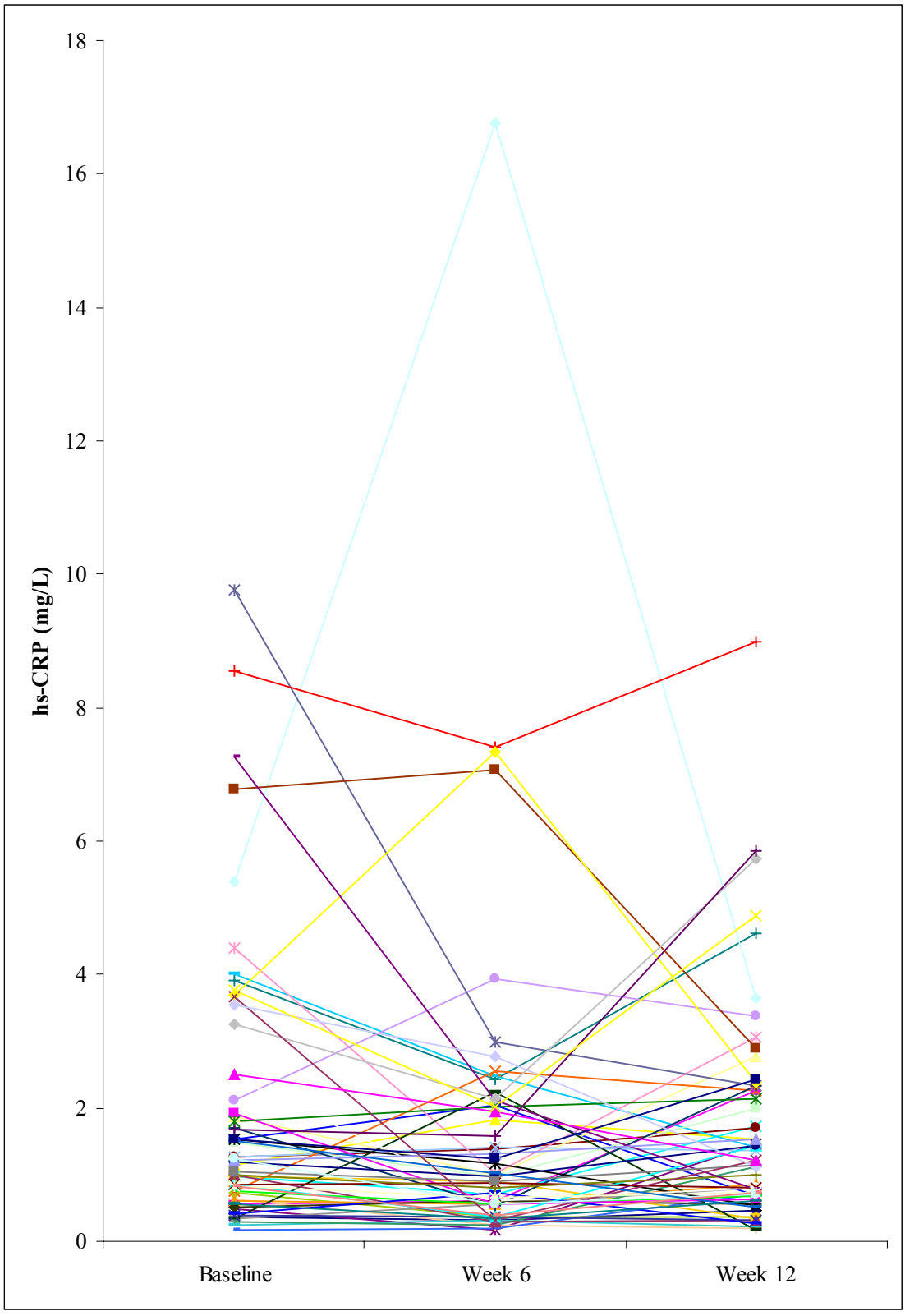

Figure 41. Effect of microcoated fenofibrate treatment on serum hs-CRP levels

2.6 Baseline PON1 concentration and activity of patients with low HDL-C levels

As summarized in table 19, the mean PON1 concentration in low HDL-C patients was 79.9 μg/mL. The median values of PON1 activity towards paraoxon, phenylacetate and diazoxon were 227.9 nmol/min/mL, 68.9 μmol/min/mL, and 7.7 μ mol/min/mL, respectively.

2.7 Effects of microcoated fenofibrate on PON1 concentration and activity in patients with low HDL-C levels

After 6 and 12 weeks of microcoated fenofibrate therapy, there were significant increase in PON1 concentration (+7.73% and +14.62%) and PON1 activity towards paraoxon (+5.69% and +10.11%), phenylacetate (+24.59% and +43.13%), and diazoxon (+8.88% and +15.27%; table 19 and figure 42). The significant greater effects of microcoated fenofibrate on PON1 levels were found at 12 weeks after treatment when compared with those effects at 6 weeks after treatment (table 19).

Table 20 and figure 43 to 48 show the correlation coefficients between the percentage change in PON1 levels and the percentage change in lipid parameters after microcoated fenofibrate therapy. The change of PON1 concentration was positively correlated with the change of HDL-C (r = 0.320 and 0.415; p = 0.012 and 0.001; figure 43) at 6 and 12 weeks, respectively. Similarly, there was significantly positive association of the change of PON1 activity towards paraoxon and the change of HDL-C (r = 0.312 and 0.475; p = 0.014 and < 0.001; figure 44) at 6 and 12 weeks, respectively. On the other hand, the significantly negative correlation between the change of PON1 activity towards paraoxon and the change of TG was observed (r = -0.342 and -0.259; p = 0.007 and 0.049; figure 45). After 12 weeks of treatment, the change of apo AI was positively correlated with the change of PON1 activity towards paraoxon (r = 0.308; p = 0.019; figure 46) and diazoxon (r = 0.305; p = 0.020; figure 47). In addition, positive association between the change of PON1 activity towards diazoxon and the change of HDL-C was also observed (r = 0.367; p = 0.005; figure 48). Although the correlation between the change of PON1 activity towards the three substrates and the change of oxLDL after microcoated fenofibrate treatment tended to be negative, this correlation did not reach statistical significance (table 20). There were

positive correlation between the change of PON1 concentration and the change of PON1 activity towards paraoxon (r = 0.509; $p < 0.001$; figure 49) and diazoxon (r = 0.281; $p = 0.033$; figure 50).

Table 19. PON1 concentration and activity towards three substrates at baseline, 6 and 12 weeks after microcoated fenofibrate treatment in patients with low HDL-C levels

PON1 levels	Baseline	6 weeks	12 weeks
Concentration (μg/mL)	79.9 ± 15.8	85.5 ± 17.5*	91.9 ± 18.6**‡
% Change▲		+7.73	+14.62
Paraoxon hydrolysis (nmol/min/mL)[a]	227.9 (77.7-371.5)	242.1 (73.6-387.5)**	257.5 (81.9-395.8)**‡
% Change▲		+5.69	+10.11
Phenylacetate hydrolysis (μmol/min/mL)[a]	68.9 (9.8-110.8)	78.9 (21.8-123.6)**	84.4 (47.3-127.2)**†
% Change▲		+24.59	+43.13
Diazoxon hydrolysis (μmol/min/mL)[a]	7.7 (2.1-16.7)	8.7 (3.7-18.5)**	8.5 (3.9-18.3)**‡
% Change▲		+8.88	+15.27

Values are shown as mean ± SD. [a]Values are shown as median (range).

▲% Change is the mean of % change from individual patient.

Significant differences from baseline are given: * $p < 0.01$ and ** $p < 0.001$

Significant differences between 6 weeks and 12 weeks after treatment are given:

† $p < 0.01$ and ‡ $p < 0.001$

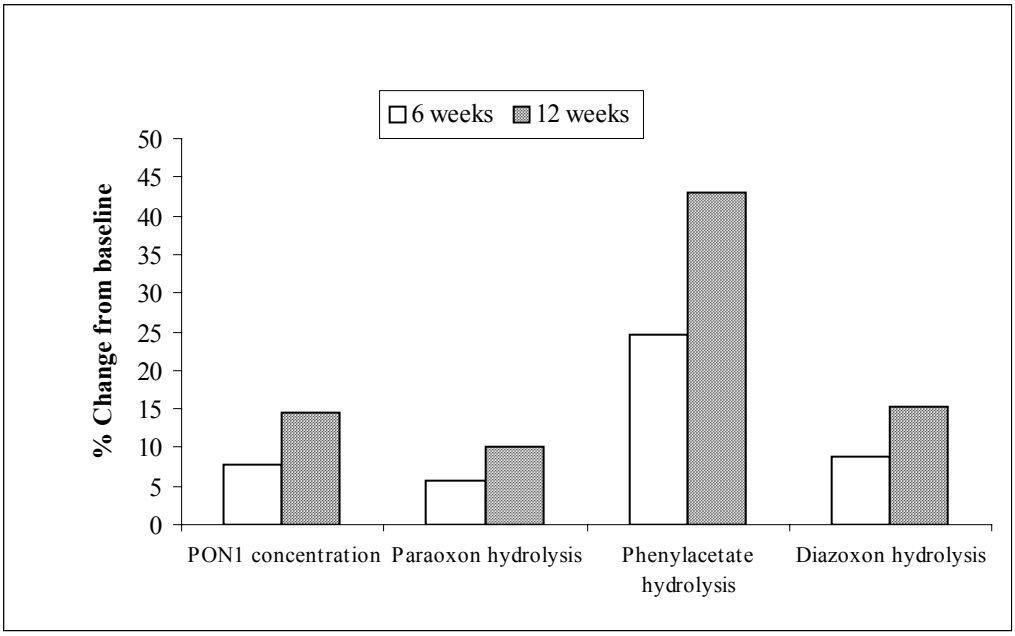

Figure 42. Percentage change from baseline of PON1 concentration and activity towards three substrates after 6 and 12 weeks of microcoated fenofibrate treatment

Table 20. Correlation coefficients between % change in PON1 levels and % change in lipid parameters after 6 and 12 weeks of microcoated fenofibrate treatment in patients with low HDL-C levels

% Change after 6 weeks	PON1 Concentration	PON1 activity towards		
		Paraoxon	Phenylacetate	Diazoxon
TC	0.108	0.014	0.039	0.100
TG	-0.237	-0.342*	0.217	0.017
HDL-C	0.320*	0.312**	0.025	0.190
LDL-C	0.122	0.101	0.035	0.042
Non-HDL-C	0.020	-0.082	0.024	0.062
OxLDL	-0.013	-0.021	-0.002	-0.101
Apo AI	0.202	0.204	0.081	0.173
Apo B	0.153	0.127	-0.006	0.101

% Change after 12 weeks	PON1 Concentration	PON1 activity towards		
		Paraoxon	Phenylacetate	Diazoxon
TC	-0.015	0.099	-0.074	0.012
TG	-0.142	-0.259*	0.109	-0.067
HDL-C	0.415**	0.475**	0.057	0.367**
LDL-C	-0.058	0.103	-0.042	-0.053
Non-HDL-C	-0.127	-0.040	-0.096	-0.069
OxLDL	-0.036	-0.057	-0.121	-0.111
Apo AI	0.220	0.308**	0.058	0.305**
Apo B	-0.028	-0.139	-0.072	-0.025

Pearson correlations are shown with the level of statistical significance where:
*$p < 0.05$ and **$p < 0.01$.

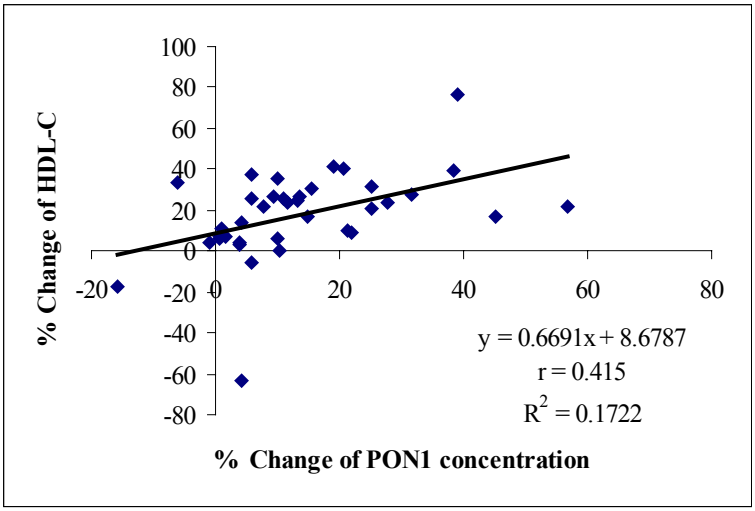

Figure 43. Relationship between % change of PON1 concentration and % change of HDL-C

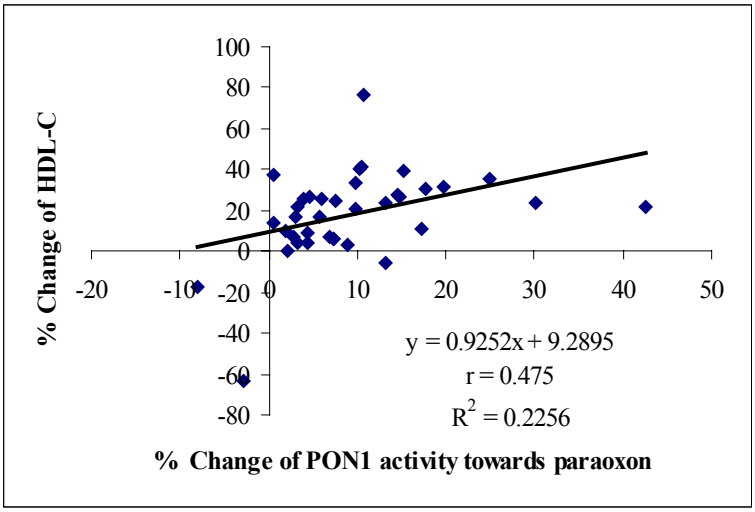

Figure 44. Relationship between % change of PON1 activity towards paraoxon and % change of HDL-C

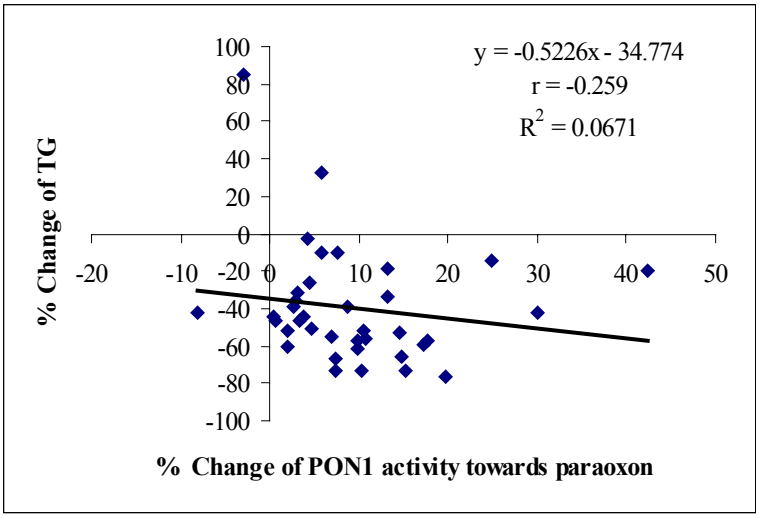

Figure 45. Relationship between % change of PON1 activity towards paraoxon and % change of TG

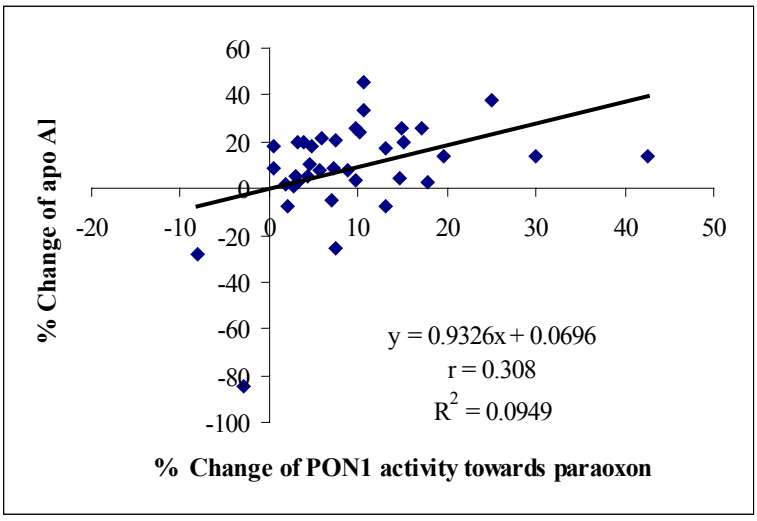

Figure 46. Relationship between % change of PON1 activity towards paraoxon and % change of apo AI

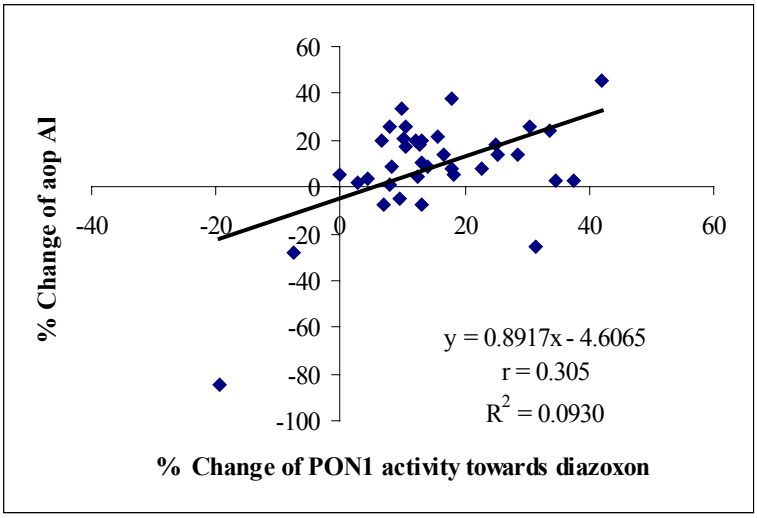

Figure 47. Relationship between % change of PON1 activity towards diazoxon and % change of apo AI

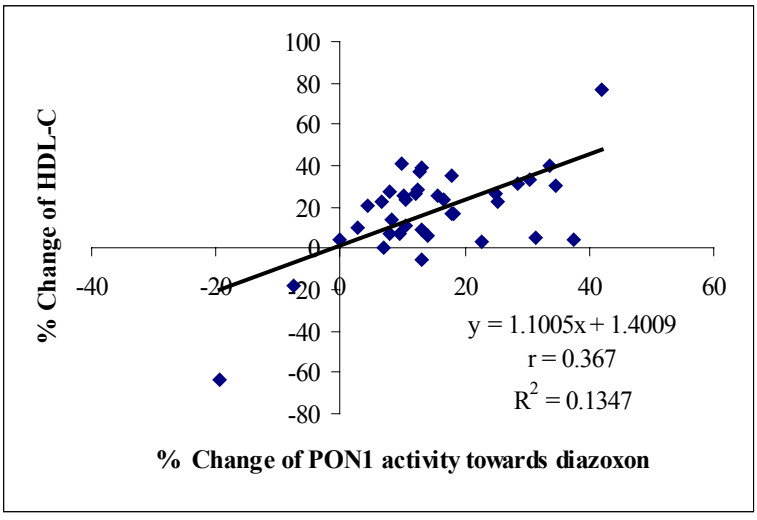

Figure 48. Relationship between % change of PON1 activity towards diazoxon and % change of HDL-C

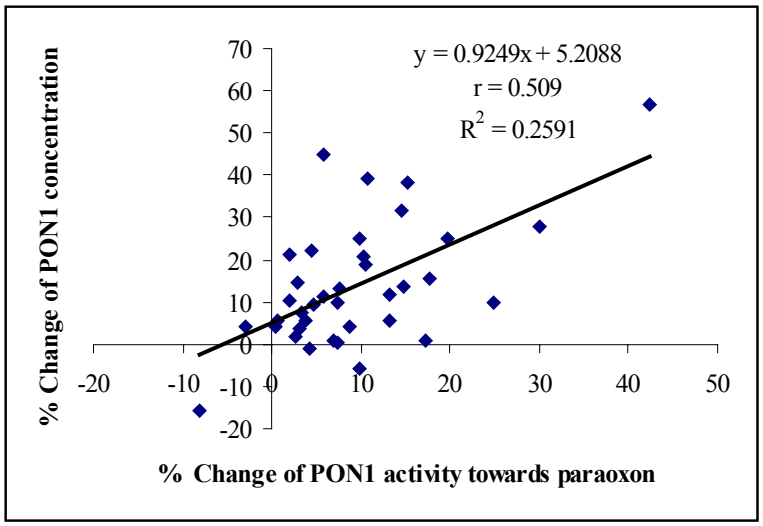

Figure 49. Relationship between % change of PON1 activity towards paraoxon and % change of PON1 concentration

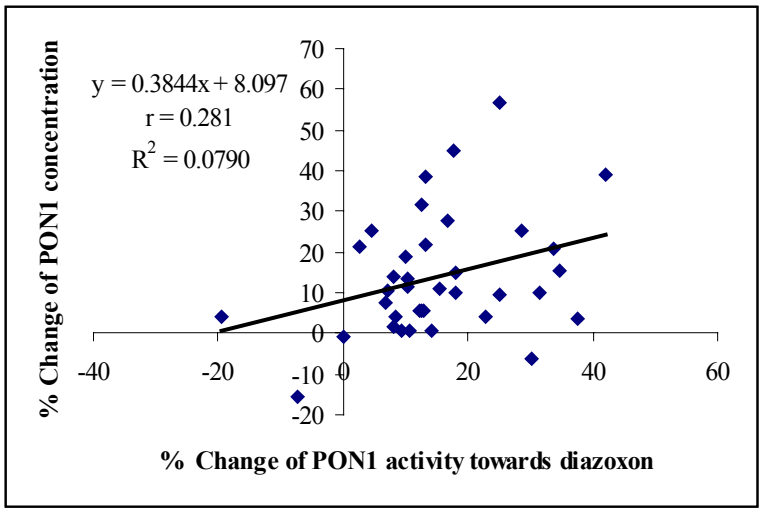

Figure 50. Relationship between % change of PON1 activity towards diazoxon and % change of PON1 concentration

2.8 Influence of the baseline TG levels on the efficacy of microcoated fenofibrate

As demonstrated in table 21, 22 and figure 51, 52, the patients were further stratified into 2 subgroups according to baseline TG levels before taken fenofibrate to investigate whether the baseline levels of TG influenced the effect of fenofibrate on lipid parameters, hs-CRP and PON1 levels. The first group included 29.3% (17/58) of patients with baseline TG levels ≤ 2.3 mmol/L, whereas the second group included 70.7% (41/58) of patients with baseline TG levels > 2.3 mmol/L. It was shown that patients with baseline TG levels > 2.3 mmol/L had a greater significance in TG reduction after 12 weeks administration of microcoated fenofibrate compared with patients with baseline TG levels ≤ 2.3 mmol/L (-49.30% vs -19.55%; $p < 0.001$; table 21). In contrast, there were no significant differences in the therapeutic response of other lipid parameters, hs-CRP and PON1 levels to microcoated fenofibrate therapy between these two subgroups according baseline TG levels ($p > 0.05$; table 21 and 22).

Table 21. Response of lipid parameters to microcoated fenofibrate therapy classified by baseline TG levels

	TG baseline ≤ 2.3 mmol/L		TG baseline > 2.3 mmol/L	
	Baseline	After 12 weeks	Baseline	After 12 weeks
TC (mmol/L)	6.03 ± 0.78	5.81± 0.98	5.86 ± 0.91	5.37 ± 0.98**
% Change▲		-2.77		-7.92
p-Value				0.190
TG (mmol/L)[a]	1.89 (1.45-2.21)	1.33 (0.99-3.80)*	3.48 (2.34-4.50)	1.61(0.80-3.77)**
% Change▲		-19.55		-49.30
p-Value				0.000
HDL-C (mmol/L)	0.93 ± 0.07	1.07 ± 0.23*	0.89 ± 0.09	1.05 ± 0.20**
% Change▲		+15.84		+17.87
p-Value				0.728
LDL-C (mmol/L)	4.13 ± 0.65	4.08 ± 0.79	3.50 ± 0.86	3.56 ± 0.84
% Change▲		+0.35		+4.60
p-Value				0.555
Non-HDL-C (mmol/L)	5.10 ± 0.73	4.74 ± 1.00	4.96 ± 0.88	4.32 ± 0.92**
% Change▲		-6.00		-12.47
p-Value				0.179
OxLDL (U/L)	60.33 ± 13.08	51.55 ± 14.32	66.58 ± 34.61	50.00 ± 15.54*
% Change▲		-10.06		-18.62
p-Value				0.280
Apo AI (g/L)	1.09 ± 0.08	1.16 ± 0.28	1.13 ± 0.13	1.20 ± 0.21*
% Change▲		+6.86		+7.41
p-Value				0.920
Apo B (g/L)	1.21 ± 0.14	1.12 ± 0.21	1.13 ± 0.21	1.02 ±0.21*
% Change▲		-6.02		-7.80
p-Value				0.703
hs-CRP (mg/L)	1.60 ± 1.72	1.50 ± 1.17	2.12 ± 2.17	1.77 ± 1.82
% Change▲		+11.61		+20.46
p-Value				0.667

Values are shown as mean ± SD. [a]Values are median (range).

▲% Change is the mean of % change from individual patient.

Significant differences from baseline within subgroup are given: * $p < 0.01$ and ** $p < 0.001$.

p-Values were obtained by comparing the differences in % change between subgroups.

Table 22. Response of PON1 levels to microcoated fenofibrate therapy classified by baseline TG levels

PON1 levels	TG baseline ≤ 2.3 mmol/L		TG baseline > 2.3 mmol/L	
	Baseline	After 12 weeks	Baseline	After 12 weeks
Concentration (μg/mL)	81.9 ± 13.6	96.7 ± 18.9**	79.1 ± 16.8	89.9 ± 18.5**
% Change▲		+15.03		+14.45
p-Value				0.899
Paraoxon hydrolysis (nmol/min/mL) [a]	257.5 (102.1-371.5)	258.7 (109.8-395.8)*	222.2 (77.7-354.2)	256.3 (81.9-384.5)**
% Change▲		+6.64		+11.55
p-Value				0.184
Phenylacetate hydrolysis (μmol/min/mL) [a]	68.9 (9.8-110.8)	83.0 (65.6-108.2)*	69.3 (30.0-106.4)	86.7 (47.3-127.2)**
% Change▲		+76.77		+29.18
p-Value				0.279
Diazoxon hydrolysis (μmol/min/mL) [a]	7.7 (2.1-16.6)	8.5 (4.7-18.3)*	7.7 (3.6-16.7)	8.5 (3.9-16.2)*
% Change▲		+20.36		+13.15
p-Value				0.362

Values are shown as mean ± SD. [a]Values are median (range).

▲% Change is the mean of % change from individual patient.

Significant differences from baseline within subgroup are given: * $p < 0.01$ and ** $p < 0.001$.

p-Values were obtained by comparing the differences in % change between subgroups.

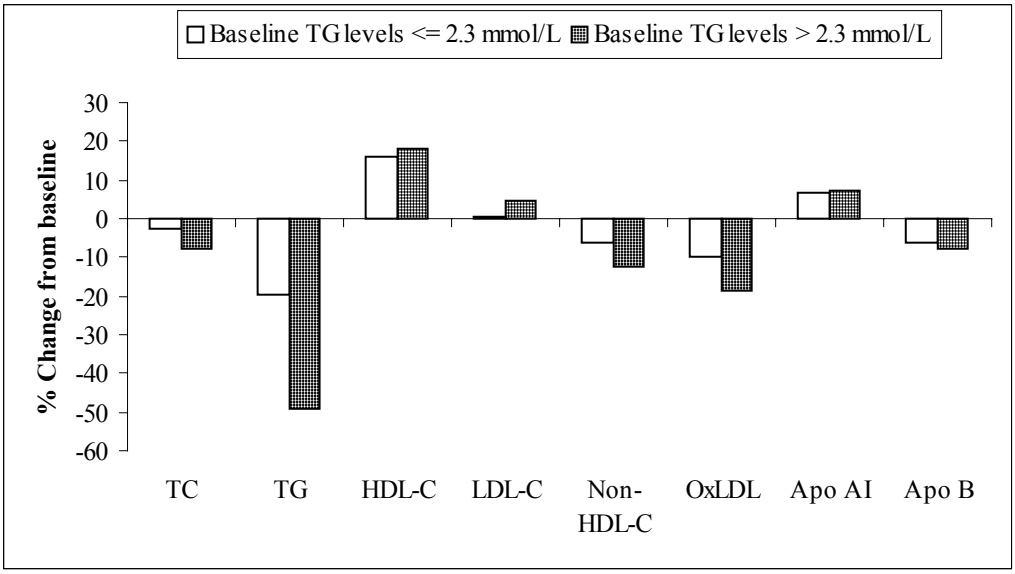

Figure 51. Percentage change from baseline of lipid parameters after 12 weeks of microcoated fenofibrate treatment according to baseline TG levels

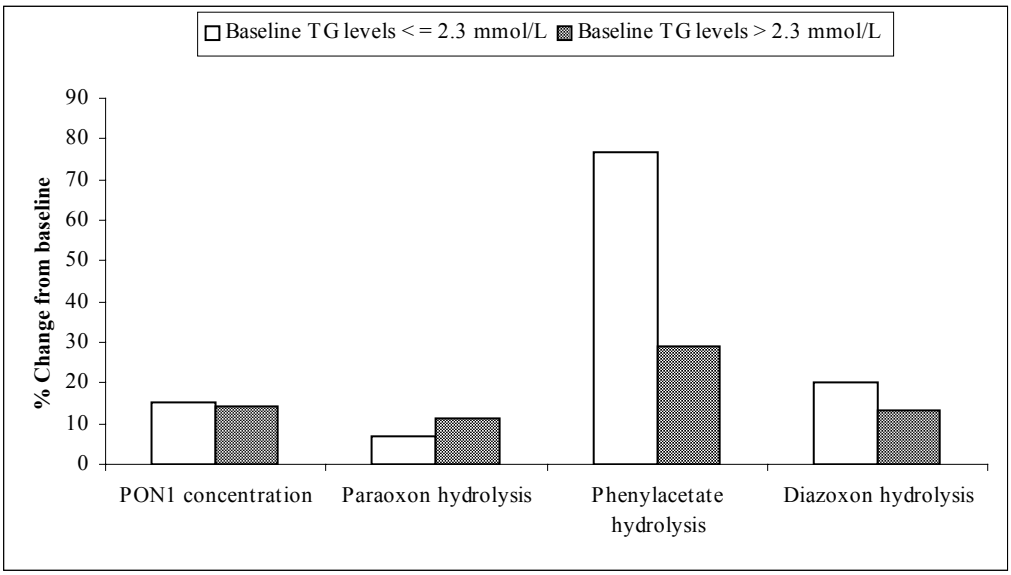

Figure 52. Percentage change from baseline of PON1 concentration and activity towards three substrates after 12 weeks of microcoated fenofibrate treatment according to baseline TG levels

2.9 Genotype and allele frequencies of the *PON1* polymorphisms in patients with low HDL-C levels

The genotype and allele frequencies of four *PON1* polymorphisms are shown in table 23. Genotype and allele frequencies for the *PON1* L55M, Q192R, T-108C and G-909C polymorphisms were 91.67%LL, 8.33%LM (L=0.96, M=0.04), 40%QQ, 50%QR, 10%RR (Q=0.65, R=0.35), 21.67%TT, 40%CT, 38.33%CC (T=0.42, C=0.58), 31.67%CC, 40%CG, and 28.33%GG (C=0.52, G=0.48), respectively. The 55LL genotype was the most common genotype found in the population (91.67%), whereas the rarest genotype was the 55MM (0%).

As reported in table 24, a good agreement was found between the observed and expected genotype frequencies at position 55, 192, -108, and -909 according to Hardy-Weinberg equilibrium (p = 0.851, 0.746, 0.390, and 0.303, respectively). These genotype frequencies did not deviate from Hardy-Weinberg equilibrium expectations using the Chi-square test. As presented in table 25, Chi-square test showed no significant linkage disequilibrium between polymorphic sites of *PON1* polymorphisms ($p > 0.05$).

Table 23. Genotype and allele frequencies of the *PON1* polymorphisms in patients with low HDL-C levels

	Polymorphic sites			
	L55M	Q192R	T-108C	G-909C
Genotype frequencies	LL : 91.67 % LM : 8.33 % MM : 0 %	QQ : 40 % QR : 50 % RR : 10 %	TT : 21.67 % CT : 40 % CC : 38.33 %	CC : 31.67 % CG : 40 % GG : 28.33 %
Allele frequencies	L : 0.96 M : 0.04	Q : 0.65 R : 0.35	T : 0.42 C : 0.58	C : 0.52 G : 0.48

Table 24. Assessment of Hardy-Weinberg Equilibrium for the *PON1* polymorphisms in patients with low HDL-C levels

		Genotype frequencies			
		Observed	Expected	Chi-square value	*p* Value
L55M	LL	55	55.296	0.145	0.851
	LM	5	4.608		
	MM	0	0.096		
Q192R	QQ	24	25.35	0.59	0.746
	QR	30	27.30		
	RR	6	7.35		
T-108C	TT	13	10.584	1.88	0.390
	CT	24	29.232		
	CC	23	20.184		
G-909C	CC	19	16.224	2.38	0.303
	CG	24	29.952		
	GG	17	13.824		

Table 25. *p* Values for pairs of the *PON1* polymorphisms demonstrating linkage disequilibrium in patients with low HDL-C levels

	p Value			
	L55M	Q192R	T-108C	G-909C
L55M	.....	0.441	0.242	0.778
Q192R		.....	0.812	0.729
T-108C			.....	0.067
G-909C				.....

Values of *p* are obtained by Chi-square test. *p* Value < 0.05 indicates linkage disequilibrium.

2.10 Influence of the *PON1* polymorphisms on baseline PON1 levels in patients with low HDL-C levels

Table 26 and 27 show serum PON1 concentration and activity towards three substrates in each genotype according to four *PON1* polymorphisms. Only the *PON1* T-108C polymorphism significantly affected serum PON1 concentration (p = 0.043; table 27). The highest PON1 concentration (82.8 μg/mL) was found in -108CC genotypes (n = 23), while the -108TT genotypes (n = 13) had the lowest PON1 concentration (70.3 μg/mL). In contrast, there were no significant differences in PON1 concentration between genotypes according to the *PON1* L55M, Q192R and G-909C polymorphisms (p = 0.516, 0.774, and 0.395, respectively; table 26 and 27).

The *PON1* Q192R polymorphism significantly influenced the variation in serum PON1 activity towards paraoxon and phenylacetate (p < 0.001 and p = 0.011; table 26). The 192RR genotypes had the highest PON1 activity towards paraoxon and phenylacetate (301.4 nmol/min/mL and 78.9 μmol/min/mL) whereas 192QQ had the lowest activity (189.3 nmol/min/mL and 63.6 μmol/min/mL) and the 192QR heterozygotes having intermediate acitivity (258.7 nmol/min/mL and 71.6 μ mol/min/mL). Meanwhile, the significant variation in PON1 activity towards diazoxon was affected by the *PON1* G-909C polymorphism (p = 0.020; table 27). The highest activity towards diazoxon was observed in the -909GG genotypes (11.5 μ mol/min/mL) and the lowest activity was found in the -909CC genotypes (5.6 μ mol/min/mL).

Table 26. Baseline PON1 concentration and activity towards the three substrates according to the *PON1* L55M and Q192R polymorphisms in patients with low HDL-C levels

PON1levels	L55M	Q192R
Concentration (μg/mL)	LL: 80.4 ± 16.4	QQ: 78.5 ± 15.1
	LM: 75.5 ± 10.3	QR: 81.5 ± 16.6
		RR: 78.5 ± 17.9
	p Value = 0.516	*p* Value = 0.774
Activity towards paraoxon[a] (nmol/min/mL)	LL: 247.4 (92.6-371.5)	QQ: 189.3 (77.7-321.0)
	LM: 202.3 (77.7-215.9)	QR: 258.7 (102.1-366.1)
		RR: 301.4 (278.3-371.5)
	p Value = 0.064	*p* Value < 0.001
Activity towards phenylacetate[a] (μmol/min/mL)	LL: 69.9 (9.8-110.8)	QQ: 63.6 (26.8-93.1)
	LM: 64.2 (46.4-79.2)	QR: 71.63 (9.8-106.4)
		RR: 78.9 (52.2-110.8)
	p Value = 0.360	*p* Value = 0.011
Activity towards diazoxon[a] (μmol/min/mL)	LL: 7.4 (2.1-16.7)	QQ: 8.9 (2.1-16.1)
	LM: 8.1 (7.2-13.6)	QR: 7.4 (4.4-16.7)
		RR: 5.1 (3.6-8.9)
	p Value = 0.640	*p* Value = 0.114

Values are shown as mean ± SD. [a]Values are median (range).

Table 27. Baseline PON1 concentration and activity towards the three substrates according to the *PON1* T-108C and G-909C polymorphisms in patients with low HDL-C levels

PON1 levels	T-108C	G-909C
Concentration (μg/mL)	TT: 70.3 ± 8.9 CT: 82.7 ± 19.6 CC: 82.8 ± 12.8 *p* Value = 0.043	CC: 80.5 ± 16.1 CG: 76.9 ± 14.9 GG: 83.9 ± 17.1 *p* Value = 0.395
Activity towards paraoxon[a] (nmol/min/mL)	TT: 215.4 (102.1-366.1) CT: 246.6 (77.7-371.5) CC: 239.7 (109.2-328.1) *p* Value = 0.530	CC: 257.5 (103.9-348.9) CG: 210.1 (92.6-366.1) GG: 247.4 (77.7-371.5) *p* Value = 0.778
Activity towards phenylacetate[a] (μmol/min/mL)	TT: 65.9 (30.0-76.0) CT: 74.9 (26.8-100.8) CC: 68.2 (9.8-106.4) *p* Value = 0.058	CC: 68.2 (9.8-100.8) CG: 70.6 (40.3-106.4) GG: 68.5 (26.8-93.1) *p* Value = 0.717
Activity towards diazoxon[a] (μmol/min/mL)	TT: 5.8 (2.1-16.) CT: 8.5 (3.6-16.7) CC: 8.4 (4.4-16.1) *p* Value = 0.229	CC: 5.6 (2.1-14.7) CG: 8.9 (3.6-16.1) GG: 11.5 (3.6-16.7) *p* Value = 0.020

Values are shown as mean ± SD. [a]Values are median (range).

2.11 Influence of the *PON1* polymorphisms on baseline lipoprotein profiles in patients with low HDL-C levels

Baseline lipoprotein profiles according to the *PON1* polymorphisms are shown in table 28 and 29. There were no significant differences in baseline lipid parameters between genotypes classified by all four *PON1* polymorphisms ($p > 0.05$).

Table 28. Baseline lipoprotein profiles according to the *PON1* L55M and Q192R polymorphisms in patients with low HDL-C levels

Parameters	L55M	Q192R
TC	LL: 5.87 ± 0.88	QQ: 5.99 ± 0.94
(mmol/L)	LM: 6.29 ± 0.79	QR: 5.84 ± 0.86
		RR: 5.96 ± 0.84
	p Value = 0.311	*p* Value = 0.818
TG[a]	LL: 2.75 (1.45-4.50)	QQ: 3.14 (1.77-4.50)
(mmol/L)	LM: 3.29 (1.86-4.50)	QR: 2.84 (1.45-4.50)
		RR: 2.45 (1.93-2.75)
	p Value = 0.259	*p* Value = 0.246
HDL-C	LL: 0.90 ± 0.09	QQ: 0.91 ± 0.09
(mmol/L)	LM: 0.95 ± 0.05	QR: 0.90 ± 0.09
		RR: 0.92 ± 0.08
	p Value = 0.248	*p* Value = 0.871
LDL-C	LL: 3.70 ± 0.86	QQ: 3.78 ± 0.90
(mmol/L)	LM: 3.93 ± 0.58	QR: 3.60 ± 0.82
		RR: 4.07 ± 0.68
	p Value = 0.563	*p* Value = 0.424

Values are shown as mean ± SD. [a]Values are median (range).

Table 28. Baseline lipoprotein profiles according to the *PON1* L55M and Q192R polymorphisms in patients with low HDL-C levels (continued)

Parameters	L55M	Q192R
Non-HDL-C	LL: 4.98 ± 0.85	QQ: 5.08 ± 0.90
(mmol/L)	LM: 5.35 ± 0.77	QR: 4.94 ± 0.82
		RR: 5.05 ± 0.80
	p Value = 0.347	*p* Value = 0.827
OxLDL	LL: 64.24 ± 30.68	QQ: 64.92 ± 19.26
(U/L)	LM: 67.55 ± 21.09	QR: 66.30 ± 38.18
		RR: 53.95 ± 14.75
	p Value = 0.815	*p* Value = 0.658
Apo AI	LL: 1.11 ± 0.11	QQ: 1.12 ± 0.12
(g/L)	LM: 1.14 ± 0.08	QR: 1.10 ± 0.11
		RR: 1.09 ± 0.09
	p Value = 0.495	*p* Value = 0.657
Apo B	LL: 1.15 ± 0.19	QQ: 1.18 ± 0.21
(g/L)	LM: 1.20 ± 0.15	QR: 1.13 ± 0.18
		RR: 1.19 ± 0.20
	p Value = 0.598	*p* Value = 0.621

Values are shown as mean ± SD.

Table 29. Baseline lipoprotein profiles according to the *PON1* T-108C and G-909C polymorphisms in patients with low HDL-C levels

Parameters	T-108C	G-909C
TC	TT: 5.78 ± 0.94	CC: 6.07 ± 0.72
(mmol/L)	CT: 5.93 ± 0.93	CG: 5.99 ± 0.91
	CC: 5.98 ± 0.79	GG: 5.63 ± 0.97
	p Value = 0.807	*p* Value = 0.276
TG[a]	TT: 0.90 (0.41-2.47)	CC: 0.85 (0.34-3.72)
(mmol/L)	CT: 0.84 (0.44-3.72)	CG: 1.00 (0.44-2.39)
	CC: 1.00 (0.34-1.92)	GG: 0.75 (0.54-2.22)
	p Value = 0.807	*p* Value = 0.308
HDL-C	TT: 0.92 ± 0.08	CC: 0.89 ± 0.09
(mmol/L)	CT: 0.90 ± 0.08	CG: 0.92 ± 0.09
	CC: 0.90 ± 0.09	GG: 0.90 ± 0.07
	p Value = 0.794	*p* Value = 0.460
LDL-C	TT: 3.56 ± 0.99	CC: 3.83 ± 0.66
(mmol/L)	CT: 3.72 ± 0.87	CG: 3.76 ± 0.83
	CC: 3.81 ± 0.74	GG: 3.53 ± 1.03
	p Value = 0.706	*p* Value = 0.542

Values are shown as mean ± SD. [a]Values are median (range).

Table 29. Baseline lipoprotein profiles according to the *PON1* T-108C and G-909C polymorphisms in patients with low HDL-C levels (continued)

Parameters	T-108C	G-909C
Non-HDL-C	TT: 4.86 ± 0.93	CC: 5.19 ± 0.69
(mmol/L)	CT: 5.02 ± 0.89	CG: 5.07 ± 0.88
	CC: 5.08 ± 0.77	GG: 4.72 ± 0.91
	p Value = 0.755	*p* Value = 0.236
OxLDL	TT: 56.54 ± 11.63	CC: 74.95 ± 46.34
(U/L)	CT: 66.77 ± 44.34	CG: 61.07 ± 14.07
	CC: 66.66 ± 14.72	GG: 57.71 ± 19.85
	p Value = 0.562	*p* Value = 0.173
Apo AI	TT: 1.13 ± 0.11	CC: 1.07 ± 0.09
(g/L)	CT: 1.13 ± 0.12	CG: 1.15 ± 0.12
	CC: 1.08 ± 0.10	GG: 1.10 ± 0.0
	p Value = 0.200	*p* Value = 0.064
Apo B	TT: 1.12 ± 0.24	CC: 1.20 ± 0.15
(g/L)	CT: 1.15 ± 0.20	CG: 1.16 ± 0.19
	CC: 1.18 ± 0.15	GG: 1.10 ± 0.23
	p Value = 0.628	*p* Value = 0.323

Values are shown as mean ± SD.

2.12 Influence of the *PON1* polymorphisms on the efficacy of microcoated fenofibrate

There was no significant difference in the change of lipid parameters and hs-CRP after microcoated fenofibrate therapy between genotypes classified by four *PON1* gene polymorphisms (data not shown).

Meanwhile, the *PON1* Q192R polymorphism significantly influenced the difference in change of PON1 activity towards paraoxon after therapy (14.98% for the 192QQ genotypes, 6.79% for the 192QR genotypes, and 3.64% for the 192RR genotypes; $p = 0.028$; table 30). The similar influence of the *PON1* T-108C polymorphism on the difference in change of both PON1 concentration (16.95% for the -108TT genotypes, 6% for the -108CT genotypes, and 3.72% for the -108CC genotypes; $p = 0.037$; table 31) and PON1 activity towards paraoxon (14.88% for the -108TT genotypes, 3.31% for the -108CT genotypes, and 2.20% for the -108CC genotypes; $p < 0.001$; table 31) was observed after treatment.

Table 30. % Change of PON1 concentration and activity towards the three substrates according to the *PON1* L55M and Q192R polymorphisms in patients with low HDL-C levels

% Change of PON1 levels▲	L55M	Q192R
Concentration	LL: 13.79 ± 15.54	QQ: 15.30 ± 16.74
	LM: 20.63 ± 15.13	QR: 13.77 ± 15.86
		RR: 13.75 ± 9.51
	p Value = 0.350	*p* Value = 0.937
Activity towards paraoxon	LL: 9.92± 13.11	QQ: 14.98 ± 15.37
	LM: 8.16 ± 4.56	QR: 6.79 ± 9.57
		RR: 3.64 ± 4.31
	p Value = 0.768	*p* Value = 0.028
Activity towards phenylacetate	LL: 44.31 ± 104.99	QQ: 39.87 ± 33.03
	LM: 36.99 ± 22.27	QR: 53.80 ± 139.76
		RR: 10.94 ± 25.92
	p Value = 0.878	*p* Value = 0.628
Activity towards diazoxon	LL: 15.58 ± 27.79	QQ: 9.98 ± 3.59
	LM: 11.69 ± 25.32	QR: 9.64 ± 3.78
		RR: 6.56 ± 1.66
	p Value = 0.765	*p* Value = 0.345

Values are shown as mean ± SD.

▲% Change is the mean of % change from individual patient.

Table 31. % Change of PON1 concentration and activity towards the three substrates according to the *PON1* T-108C and G-909C polymorphisms in patients with low HDL-C levels

% Change of PON1 levels▲	T-108C	G-909C
Concentration	TT: 16.95 ± 18.63	CC: 12.38 ± 16.70
	CT: 6.00 ± 11.97	CG: 13.24 ± 16.01
	CC: 3.72 ± 15.26	GG: 18.30 ± 13.46
	p Value = 0.037	*p* Value = 0.492
Activity towards paraoxon	TT: 14.88 ± 15.52	CC: 8.63 ± 9.91
	CT: 3.31 ± 5.60	CG: 10.99 ± 15.05
	CC: 2.20 ±7.75	GG: 9.26 ± 11.96
	p Value < 0.001	*p* Value = 0.828
Activity towards phenylacetate	TT: 33.72 ± 35.12	CC: 60.21 ± 170.101
	CT: 14.38 ± 37.39	CG: 26.83 ± 26.38
	CC: 30.78 ± 57.58	GG: 49.27 ± 54.91
	p Value = 0.353	*p* Value = 0.561
Activity towards diazoxon	TT: 21.83 ± 39.47	CC: 7.41 ± 2.57
	CT: 5.58 ± 17.24	CG: 10.19 ± 3.60
	CC: 4.95 ± 13.34	GG: 10.69 ± 3.92
	p Value = 0.075	*p* Value = 0.312

Values are shown as mean ± SD.

▲% Change is the mean of % change from individual patient.

2.13 Effect on anthropometric parameters and blood pressure

Anthropometric parameters and blood pressure at each visit are demonstrated in table 32 and figure 53. There were significant reductions in the average values of waist circumference (-1.47% and -1.16%), WHR (-1.13% and -0.95%), BW (-1.32% and -1.07%), BMI (-1.29% and -1.17%) at 6 and 12 weeks of microcoated fenofibrate treatment, respectively. While significant decrements of mean SBP (-3.6%) and DBP (-2.99%) were observed after 12 weeks of treatment.

2.14 Drug compliance

Majority of patients complied well with the study medication. Ninety percent of the patients had taken an average of 90% to 100% of the study medication. Mean compliance to drug treatment at 6 and 12 weeks were 97.13% and 95.40%, respectively.

Table 32. Anthropometric parameters and blood pressure at baseline, 6 and 12 weeks after microcoated fenofibrate treatment in patients with low HDL-C levels

	Baseline	6 weeks	12 weeks
Waist circumference (cm)	92.16 ± 7.12	90.80 ± 6.99**	91.38 ± 6.93**
% Change▲		-1.47	-1.16
WHR	0.90 ± 0.04	0.89 ± 0.04**	0.89 ± 0.04**
% Change▲		-1.13	-0.95
BW (kg)	74.58 ± 9.99	73.59 ± 9.92**	74.15 ± 9.75**
% Change▲		-1.32	-1.07
BMI (kg/m^2)	26.3 ± 3.0	25.9 ± 3.0**	26.1 ± 3.0**
% Change▲		-1.29	-1.17
SBP (mmHg)	126.3 ± 12.6	124.2 ± 12.9	121.4 ± 12.1*
% Change▲		-1.47	-3.60
DBP (mmHg)	73.7 ± 9.8	73.1 ± 8.3	71.1 ± 8.2*
% Change▲		+0.22	-2.99

Values are shown as mean ± SD. ▲% Change is the mean of % change from individual patient.
Significant differences from baseline are given: * $p < 0.01$ and ** $p < 0.001$.

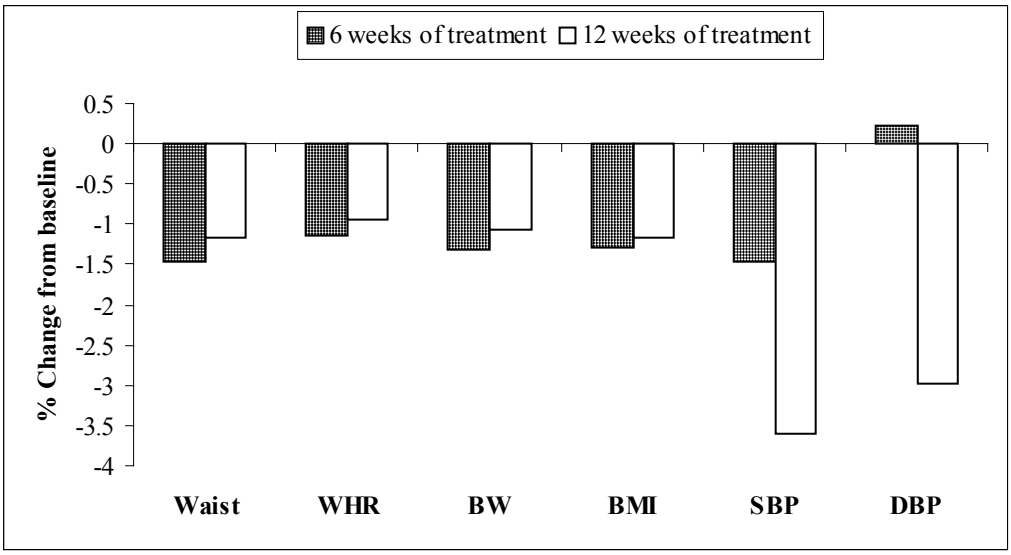

Figure 53. Percentage change from baseline of anthropometric parameters and blood pressure after 6 and 12 weeks of microcoated fenofibrate treatment

2.15 Tolerability and safety profiles

Safety parameters and adverse events are summarized in table 33. Microcoated fenofibrate treatment was well tolerated in this study. Fifty-eight of 61 patients (95.1%) completed the study. The withdrawal rate in this study was 4.9% (3/61). After 6 weeks of therapy, two patients withdrew due to adverse events (rash) and one patient discontinued treatment due to loss of follow up. There were changes in the levels of AST (+28.84% and +8.66%), ALT (+15.23% and –0.31%) and CK (+41.01% and +36.52%) after 6 and 12 weeks of treatment, respectively. However, neither of the patients with transaminases ≥ 3 times ULN nor CK ≥ 5 times ULN were observed. During the study adverse events were reported by a total of 11 patients (18%). None of the patients experienced serious adverse events. The most frequently reported adverse events were those affecting the gastrointestinal system (8%) including nausea, dyspepsia and flatulence, and the metabolic/ nutritional system (8%) including stimulating appetite, loss of appetite and sweating (table 33).

Table 33. Safety parameters and adverse events after microcoated fenofibrate treatment in patients with low HDL-C levels

	Baseline	6 weeks	12 weeks
AST (U/L)	26.20 ± 6.52	32.43 ± 16.59*	27.29 ± 8.88
% Change▲		+28.84	+8.66
ALT (U/L)	29.74 ± 10.58	33.70 ± 22.73	28.53 ± 16.13
% Change▲		+15.23	-0.31
CK (U/L)	183.93 ± 90.32	249.97 ± 160.59**	233.03 ± 143.63**
% Change▲		+41.01	+36.52
Increased AST ≥ 3 ULN (n)		0	0
Increased ALT ≥ 3 ULN (n)		0	0
Increased CK ≥ 5 ULN (n)		0	0
Adverse events, n (%)			
Gastrointestinal events: nausea, dyspepsia, flatulance			5 (8%)
Metabolic/ nutritional events: stimulating appetite, loss of appetite, sweating			5 (8%)
Skin/ appendages: rash, dry skin			3 (5%)

▲% Change is the mean of % change from individual patient.

Significant differences from baseline are given: * $p < 0.01$ and ** $p < 0.001$.

CHAPTER V
DISCUSSION

The discussion of this study was divided into 2 parts as follows:

Part 1: The study of PON1 status in healthy Thai population

Part 2: The study of effect of fenofibrate therapy on PON1 status in patients with low HDL-C levels

Part 1: The study of PON1 status in healthy Thai population

PON1 is able to hydrolyze a variety of substrates, including paraoxon, diazoxon, and phenylacetate (32, 197). As yet, all of the physiological substrates of PON1 are unknown but recent studies suggest that PON1 prevents LDL oxidation by hydrolyzing lipid peroxides in the lipoprotein (25, 122, 127). PON1 also hydrolyses homocysteine thiolactone, a proatherogenic metabolite (200) and PAF (201). However, there has been some controversy over whether PON1 activity, as measured by synthetic substrates, will reflect the antioxidant capacity of this enzyme (202). A study from Aviram *et al.* indicated that the PON1 active site required for its protective role against LDL oxidation were not identical to those needed for its PON1 activity towards paraoxon and phenylacetate (122). Although PON1 activity towards synthetic substrates and its protection against LDL oxidation do not involve exactly the same active site, these activities are still related (37).

In this study, serum PON1 activity towards paraoxon was more positively correlated with HDL-C and apo AI concentrations than activity measured by phenylacetate and diazoxon. This was in agreement with other studies in which positive associations between PON1 activity towards paraoxon and HDL-C as well as apo AI were observed (197, 203). Thus, it is likely that paraoxon seemed to be better surrogate substrates than phenylacetate and diazoxon for measuring PON1 activity. Jarvik *et al.* found that the PON1 activity towards diazoxon was more predictive of vascular disease status than was the PON1 activity towards paraoxon in marginal

analysis (143). However, in the only prospective study published so far, paraoxon but not diazoxon hydrolysis was an independent risk factor for CHD events (144).

The higher mean PON1 concentration was found in female compared with male. Whereas, no gender differences in serum PON1 activity towards the three substrates were observed in this study, even though serum PON1 activity was positively correlated with HDL-C and there was a significant difference in HDL-C concentration between males and females as has been reported previously (198). In addition, no correlation was found between serum PON1 activity towards three substrates and other demographic variables, including age, WHR, BMI, SBP, DBP as well as FBG. Meanwhile, PON1 concentration positively correlated with PON1 activity towards paraoxon and negatively correlated with WHR, SBP and DBP.

Interestingly, the concentration of circulating oxLDL which might reflect oxidative conditions *in vivo*, was not significantly correlated with serum PON1 activity towards the three substrates in this healthy Thai subjects. In this respect, it is likely that PON1 is not the only antioxidant in HDL subfraction in the blood. Moreover, other components may have a greater impact in preventing LDL oxidation or destroying specific oxidized lipoprotein constituents in the circulation. However, purified PON1 has been proved to prevent the oxidative modification of LDL (16, 24, 25) and to protect HDL against oxidation (125) *in vitro*.

In this study, we found that the serum PON1 activity among individuals with the same genotype differed. This finding is in agreement with the other studies that there was at least a 13 fold variation in PON1 activity among individuals with the same genotype (33, 35, 204). It is possible that not only the serum PON1 activity is influenced by the *PON1* polymorphisms but also by the other factors. We found that the serum PON1 activity towards paraoxon, but neither phenylacetate nor diazoxon, was positively correlated with HDL-C and apo AI concentrations. Therefore, HDL-C and apo AI concentrations are likely to be one of the several factors that account for the variation in serum PON1 activity. Additionally, the effects of environmental factors such as diet and exercise on serum PON1 activity have also been reported by several studies (148, 152, 158, 205).

Based on the results of correlation of various lipid parameters in this part, the inverse relation in order between HDL-C and oxLDL, TG, apo B, non-HDL-C and

LDL-C were found, supporting consistent protective effects of HDL-C on atherogenic lipid particles (14, 25, 72, 206). As compared with LDL-C, non-HDL-C had better association with apo B, oxLDL, TG and HDL-C. Among the correlation between oxLDL and various lipid parameters, non-HDL-C had the strongest correlation. Taken together, it is likely that non-HDL-C is better marker of atherogenic lipid particles than LDL-C since it encompasses not only LDL-C but also TG-rich lipoproteins (11).

In this investigation, we found that the observed distributions of all PON1 genotype frequencies were in Hardy-Weinberg equilibrium, indicating that a bias due to population sampling was ruled out. The L and M allele frequencies for the *PON1* coding-region (L55M) polymorphism were similar to those of Japanese and Chinese populations who displayed very low frequencies of the M allele (M=0.06) (133, 207, 208), but these results were different from those of Asian Indian and Caucasian populations (M=0.36) (47, 132, 209). However, differences in Q and R allele frequencies for the *PON1* Q192R polymorphism between Thai population (Q=0.71, R=0.29) and Japanese population (Q=0.40, R=0.60) were observed, but were not found when compared with those of Caucasian populations (Q=0.74, R=0.26) (47, 133, 207). The very low frequency of the 55M genotype in the Thai population was surprising. However, in a recent study of Han Chinese, no polymorphism of this site was found (210). The allele frequencies for *PON1* regulatory-region (T-108C and G-909C) polymorphisms also differed from those observed in both Japanese and Caucasian populations (47, 132, 133). Our data support the other previous studies that there are interpopulation differences in allele frequencies for the *PON1* polymorphisms (47, 132, 133, 208). This may be a coincidence, or selection pressure may have acted on these polymorphisms to maintain specific allele frequencies across different ethnic groups. Five *PON1* regulatory polymorphisms at position -108, -126, -162, -832 and -909 have been identified by DNA sequence analysis (134). Three of these polymorphisms at position -108, -162 and -909 were found to have effects on PON1 expression levels in population study (47). In our study, only position -108 and -909 were analyzed because of the limitation in position –162 analysis.

We found significant linkage disequilibrium between Q192R and L55M, Q192R and T-108C, and Q192R and G-909C as has been previously reported but in different pattern (47, 134). In this respect, it should be noted that there was interracial

variability in the allele frequencies for *PON1* polymorphisms as well as the different linkage disequilibrium patterns between the *PON1* polymorphisms. Additionally, it is possible that any of the impacts of the *PON1* polymorphisms on the variation in serum PON1 levels are, at least in part, due to linkage disequilibrium with the other polymorphic sites. The implication of these differences may relate to the difference in susceptibility to cardiovascular diseases among ethnic groups. Since several studies have identified *PON1* gene as an independent genetic risk factor for CHD (38, 39, 41, 207), although there was no complete agreement on this point (136, 208, 211, 212).

As expected, there were significant variations in both serum PON1 concentration and activity as a function of *PON1* polymorphisms. In our study, we found that only the *PON1* L55M polymorphism was associated with significant variation in the serum PON1 concentration. This result agrees with the previous study that the 55L allele produced more PON1 mRNA and that 55LL genotypes had significantly more PON1 concentration than 55MM carriers (198, 213). Significant variations in serum PON1 activity among genotypes classified by the *PON1* Q192R and G-909C polymorphisms were observed when paraoxon was used as substrate. Likewise, the variations in serum PON1 activity among these genotypes were also observed when diazoxon was used as substrate but in opposite direction. No significant variation in serum PON1 activity using phenylacetate as substrate was observed among *PON1* genotypes classified by all four *PON1* polymorphisms. From this observation, paraoxon and diazoxon appeared to be discriminating substrates, while phenylacetate appeared to be a non-discriminating substrate with respect to the *PON1* polymorphisms as reported from the former (214). The effect of *PON1* Q192R polymorphism on serum PON1 activity towards paraoxon was similar to that reported in the previous studies (196, 198). These findings indicate a complex interplay between the *PON1* polymorphisms and activity towards different substrates. Surprisingly, the *PON1* T-108C polymorphism did not significantly affect the variation in both PON1 concentration and activity towards any substrates in healthy Thai population. These findings contradict the results reported by Brophy *et al.* that individuals with –108TT genotypes had the lowest PON1 activity towards phenylacetate, while those with –108CC genotypes had the highest activity (47). In addition, they noted that the T-108C polymorphic site appeared to have a dominant

effect on PON1 expression while the G-909C polymorphic site appeared to have a minor contribution to transcriptional regulation (47). Furthermore, Suehiro *et al.* reported that individuals with the –108CC genotypes had about 1.2 fold higher PON1 concentration than those with –108TT genotypes (133). These differences could be ethnically based. Despite the T-108C polymorphic site was not the functional polymorphism of PON1 levels in our population, it was in linkage disequilibrium with Q192R polymorphic site.

To explore which of the factors significantly explained the variation in serum PON1 levels, we fitted a multiple linear regression model that selected only the most influential variable. Stepwise multiple regression analysis indicated that in this population the *PON1* L55M polymorphism was identified as a significant contributor to PON1 concentration. The significant contributors for PON1 activity towards paraoxon were the *PON1* Q192R polymorphism and PON1 concentration. While both *PON1* G-909C and Q192R polymorphisms contributed to the variation in PON1 activity towards diazoxon. Besides, all four *PON1* polymorphisms were unable to find as contributor to PON1 activity towards phenylacetate, supporting that phenylacetate appeared to be a non-discriminating substrate of PON1 with respect to the *PON1* polymorphisms. On the contrary, Brophy *et al.* found that both *PON1* T-108C and L55M polymorphisms accounted for 23% and 5%, respectively, to PON1 activity towards phenylacetate in Caucasians (47).

The data presented in this study indicates that the *PON1* Q192R and G-909C polymorphisms significantly influenced on the variation in TG, HDL-C and apo AI in the healthy Thai population. PON1 could be involved in lipid metabolism, as this enzyme probably hydrolyzes multiple oxygenated forms of polyunsaturated fatty acids of LDL-associated phospholipids (25, 122, 127). Our data are in agreement with those reported by some studies (215, 216) but not others (217). In this respect, it should be noted that the impact of the *PON1* polymorphisms on lipid profiles may be variable depending on the ethnicity of the study population.

Our data demonstrate that the physiological relevance of the *PON1* polymorphisms in the Thai population is that they are associated with significant differences in serum PON1 concentration and activity. The influence of the *PON1* polymorphisms on the variation in serum PON1 activity is substrate-dependent.

Additionally, not only do allele frequencies of *PON1* polymorphisms vary between different ethnic populations, but also the linkage disequilibrium patterns between the *PON1* polymorphisms differ among ethnic populations. This study thus firmly establishes a genetic basis for the variation in serum PON1 levels. Finally, our study provides the first baseline data of PON1 status in the Thai population, which differs significantly from other oriental populations.

Part 2: The study of effect of microcoated fenofibrate therapy on PON1 status in patients with low HDL-C levels

In this study, 6 and 12 weeks administration of microcoated fenofibrate significantly lowered TC (6.41% and 6.41%), TG (34.63% and 40.58%), non-HDL-C (10.09% and 10.58%), oxLDL (13.35% and 16.11%), and apo B (8.15% and 7.28%), respectively. On the contrary, HDL-C and apo AI levels were significantly increased by 14.68% and 6.89% after 6 weeks, and 17.28% and 7.25% after 12 weeks of treatment, respectively. As expected, the major effects of microcoated fenofibrate in reducing TG and increasing HDL-C and apo AI concentrations, which are consistent with its mechanism via the activation of the PPAR α (101-104), were observed in this study. Fibrates increase the expression of lipoprotein lipase (LPL), which catalyzes the hydrolysis of TG, and also decrease the expression of apo CIII gene resulting in the reduction of apo CIII mRNA and apo CIII levels (101-103). Since apo CIII is regarded as an inhibitor of LPL, these actions may be central in the TG-lowering properties of fenofibrate. Meanwhile, apo AI mRNA and apo AI levels are increased by fibrates (101-103), supporting the hypothesis that a direct action of fibrates on apo AI gene expression results in increasing apo AI and HDL-C concentrations. Apo AI is the major apolipoprotein of HDL particle (102, 103).

The effects of microcoated fenofibrate in lowering of TG levels and increasing of HDL-C levels obtained from our study were similar to those described with some studies (194), but less than those studies for the effects on TC and LDL-C (194) levels. Therefore, the difference of these results might be explained on the basis of the distinction in the severity of dietary control, genetic effect, patient behaviour and disease state of patient.

During the process of LPL-mediated lipolysis, there is a shift in the size distribution pattern of LDL from smaller and more dense of LDL particles to particles which are larger, less dense, and less subject to oxidation (218). Recent studies demonstrated that fenofibrate treatment significantly increased LDL particle size and the change in LDL particle size was significantly associated with changes in TG, TC, apo B, and LDL/apo B ratio but not with the change in LDL-C concentration (219, 220). It is notable that its change in LDL particle size may occur without a reduction in LDL-C and even in the presence of an increase in LDL-C concentrations (218, 220). Thus, the significant reduction of both apo B and non-HDL-C, without significant reduction of LDL-C found in our study after microcoated fenofibrate treatment, may be indicative of a beneficial increase in LDL particle size and a reduction in the number of circulating LDL particles, which could reduce the atherogenesis process. Apo B concentration is a crude marker of the number of atherogenic particles and reflects the number of LDL particles as well as small dense LDL particles (221). Additionally, non-HDL-C is an estimate of all atherogenic lipid particles (221). Several clinical studies have shown that non-HDL-C is a strong and independent predictor of cardiovascular events (222-224). Cui *et al.* demonstrated that non-HDL-C was a better predictor of cardiovascular mortality than LDL-C during an average follow-up of 19 years in dyslipidemic patients (224). Nevertheless, ATPIII recommended LDL-C as a primary target of cholesterol-lowering therapy while non-HDL-C was defined as a secondary target of treatment in patients with high TG after achieving LDL-C target (11).

According to ATPIII, a low HDL-C is defined as a level of < 40 mg/dL whereas a level of ≥ 60 mg/dL is classified as a high HDL-C level as well as negative risk factor of CHD (11). Based on the results of large-scale epidemiological studies, it has been reported that a low HDL-C level is an independent risk factor for CHD (1-5, 69). Every 1 mg/dL increase in HDL-C is associated with CHD risk reduction of 2% in men and 3% in women (69). It has been reported that the most important consideration in clinical practice is not the percent increase in HDL-C, but the HDL-C level achieved on therapy (225). Hence, it is justified to consider the proportion of patients who achieve HDL-C level of ≥ 40 mg/dL, a cut-off value that has been suggested to define low HDL-C level. In our study, 47.5% (29/61) and 55.2% (32/58)

of patients with baseline low HDL-C levels (< 40 mg/dL) achieving HDL-C treatment goal of ≥ 40 mg/dL after 6 and 12 weeks of microcoated fenofibrate therapy, respectively. However, none of patients achieved HDL-C ≥ 60 mg/dL which is the value categorized as negative risk factor of CHD was observed after microcoated fenofibrate therapy.

Interestingly, the significant reduction in circulating oxLDL was found after microcoated fenofibrate treatment despite the nonsignificant change in LDL-C levels. There was significantly negative correlation between the change of oxLDL and the change of HDL-C ($r = -0.453$; $p < 0.01$). Furthermore, the correlation between the change of oxLDL and the change of apo AI and PON1 activity tended to be negative but this correlation did not reach statistical significance. Therefore, in view of these results, it seems reasonable to postulate that oxLDL reduction after fenofibrate treatment could be partly a consequence of increasing HDL-C and apo AI concentrations and / or increasing PON1 activity. Another possibility is fenofibrate may induce change in LDL particle size from smaller and more dense to larger and less dense which is less susceptible to oxidation resulting in the reduction of oxLDL.

This study had the limitations of being open-label, nonrandomized, and not placebo controlled. Nevertheless, the same trends of data which have been confirmed by other double-blinded and controlled trials (226, 227) have indicated that fenofibrate is effective across a wide range of dyslipidemic states.

CRP is an extremely sensitive, nonspecific, acute-phase reactant produced in response to most forms of tissue injury, infection, and inflammation and regulated by cytokines, including interleukin-6 (IL-6), IL-1 and TNF-α (49). Clinical guidelines have recently been published indicating that CRP levels of < 1, 1-3 and > 3 mg/L correspond to low, moderate and high risk for future vascular events, respectively (50, 53). After microcoated fenofibrate therapy, there was no significant difference in the mean hs-CRP when compared with those at baseline. This result is in contrast to those reported in the previous study that fenofibrate led to a marked reduction of CRP levels in combined hyperlipidemic patients with relatively high baseline CRP levels (> 10 mg/L) (219). A significant decrease of hs-CRP levels in combined hyperlipidemic patients with low to moderate baseline hs-CRP after fenofibrate was also reported by Wang *et al.* (227). In contrast to another study, treatment with fenofibrate in

hypertriglyceridemic patients with low to moderate baseline hs-CRP had no significant effect on hs-CRP reduction compared with placebo (228). This discrepancy may be partly due to the difference in baseline hs-CRP levels. However, the effect of fenofibrate on the reduction of hs-CRP was found only in the patients with baseline hs-CRP > 3 mg/L after 12 weeks of therapy. Additionally, 53.9% (7/13) of patients with baseline hs-CRP levels of > 3 mg/L achieved hs-CRP levels of < 3 mg/L after fenofibrate therapy in our study. It is likely that fenofibrate may effectively lower hs-CRP only among patients with elevated baseline levels. This beneficial effect may have an impact on the prevention of CHD events by fenofibrate.

There is considerable interest in the potential pharmacological effects of various antidyslipidemic drugs on PON1 activity. Of the lipid-lowering drugs, simvastatin, gemfibrozil and micronized fenofibrate therapy have been reported to raise PON1 activity in patients with familial hypercholesterolaemia, hyperlipidemia and CHD, respectively (63-65). However, this effect was not found in bezafibrate and gemfibrozil therapy in patients with type IIb hyperlipoproteinaemia, and ciprofibrate therapy in patients with familial hyperlipoproteinaemia (66, 67). Our study shows that in dyslipidemic patients with low HDL-C levels, microcoated fenofibrate increased both PON1 concentration and activity towards the three substrates. In the recent study, fenofibric acid was found to induce *PON1* gene-promoter activity (169). *In vitro* exposure of human hepatoma cells to fenofibric acid caused a 50% and 30% increase in PON1 activity and PON1 mRNA, respectively (169). This finding may explain the effect of fenofibrate in increasing PON1 concentration significantly. The increased PON1 activity by fenofibrate may be due to increase PON1 concentration. In our study, the positive correlation between the change of PON1 concentration and the change of PON1 activity towards paraoxon was found ($r = 0.509$; $R^2 = 0.259$; $p < 0.001$). Thus, approximately 25.9% of the increase in PON1 activity by fenofibrate treatment was attributable to increase in PON1 concentration. Other contributing factors in increasing PON1 activity may be the increase in HDL-C and apo AI concentrations. This effect may contribute to increase PON1 activity since PON1 has been described as preferentially associated with HDL subfractions containing apo AI (28, 35, 120). The induction of the *PON1*-gene expression by fenofibric acid does not seem to be a class effect, since it was demonstrated that gemfibrozil has no inducing

effect on the *PON1* gene promoter in human hepatoma cells (169). This *in vitro* data correlated with the clinical effect of gemfibrozil therapy in patients with type IIb hyperlipoproteinaemia which revealed no effect on PON1 activity (66). However, Paragh *et al.* reported that PON1 activity in patients with hyperlipidaemia was increased after gemfibrozil treatment (64).

Despite PON1 activity has been shown to be substrate-dependent and may be quite opposite for different substrates (33, 35, 123), the significantly increased in PON1 activity across various three substrates including paraoxon, phenylacetate and diazoxon was observed after fenofibrate treatment in our study. Nevertheless, Tomas *et al.* reported that serum PON1 activity towards paraoxon was significantly increased after simvastatin treatment but there was a nonsignificant trend to increase PON1 activity towards phenylacetate after therapy (63). The basis of the distinction in mechanism of action of drug, genetic effect and disease state of patient may be taken to clarify this difference.

The results of the analyses stratified by baseline TG levels indicate that the effect of fenofibrate treatment on the TG reduction was dependent of baseline TG levels (Table 24). The greater baseline of TG levels, the greater reduction in TG was achieved. This finding is consistent with the previous study which demonstrated that TG-lowering effects of fenofibrate were positively related to baseline TG levels (186, 193). On the contrary, baseline HDL-C levels were inversely related to the effect of fenofibrate on the TG reduction (186, 193, 227), whereas the therapeutic responses of other lipid parameters and PON1 levels to fenofibrate treatment were significantly independent of the baseline TG levels in our study. It is of interest that despite the effect of fenofibrate treatment on the oxLDL reduction was nonsignificantly dependent of baseline TG levels, the percentage change in oxLDL reduction after therapy tended to be higher in patients with baseline TG levels > 2.3 mmol/L compared with patients with baseline TG levels ≤ 2.3 mmol/L (-18.62% vs –10.06%; $p = 0.280$). It is likely that the greater reduction in TG contributed to the decrease in the oxidation process of LDL. It has been established that the reduction of TG was associated with the increase in large buoyant LDL particle (218, 219). Hence, the TG reduction also contributed to the reduction of oxLDL together with increasing in HDL-C, apo AI and PON1 levels.

Our study revealed that only the *PON1* T-108C polymorphism was associated with baseline PON1 concentration in patients with low HDL-C levels. This finding is in agreement with the report from Suehiro *et al.* (133). The -108CC genotypes had higher PON1 concentration than the -108TT genotypes. As expected, the *PON1* Q192R polymorphism affected baseline PON1 activity towards paraoxon. This observation was similar to that shown in the previous studies (33, 35). Additionally, the *PON1* G-909C polymorphism influenced on the variation of baseline PON1 activity towards diazoxon. The results presented in this study indicate that none of *PON1* polymorphisms significantly affected the difference of baseline lipid parameters in low HDL-C patients. Our data are in agreement with those reported by some studies (38, 207, 212) but not others (215, 216). This discrepancy might be explained on the basis of the distinction in the study population. However, because the sample size of each genotype group in this part of study was relatively small, these results should be viewed with caution.

Comparing between healthy subjects and patients with low HDL-C levels, there were the differences of the allele frequencies of *PON1* T-108C and G-909C polymorphisms between these two groups while the allele frequencies of *PON1* L55M and Q192R polymorphisms were similar. Our results revealed that *PON1* L55M polymorphism influenced the variation of PON1 concentration in healthy subjects whereas this variation was affected by *PON1* T-108C polymorphism in patients with low HDL-C levels. In addition, the low levels of PON1 concentration, its activity as well as HDL-C level were found in patients with low HDL-C levels compared with those of healthy subjects. In these contexts, the particular function of PON1 may be relevant differently. *PON1* T-108C polymorphism may partly contribute to the low concentration of PON1. However, the sample size in patients with low HDL-C levels was not enough for genotype comparisons.

The number of previous intervention studies on the relationship between *PON1* genotypes and lipid parameters is very limited. Tomas *et al.* reported that the therapeutic response of lipid parameters and PON1 activity to simvastatin therapy was independent of the *PON1* L55M and Q192R polymorphisms (63). In contrast, these *PON1* polymorphisms modified the effect of pravastatin on HDL-C and apo AI concentrations in another study (229). Individual with 55LL homozygotes and 192R

allele carriers had statistically significantly greater HDL-C and apo AI changes after pravastatin treatment (229). Our study found no significant differences in the therapeutic response of lipid parameters after fenofibrate treatment between genotype groups classified by the *PON1* L55M, Q192R, T-108C and G-909C polymorphisms. However, the therapeutic response of PON1 activity towards paraoxon was dependent of the *PON1* Q192R and T-108C polymorphisms. In addition, the *PON1* T-108C polymorphism also influenced on the difference in the change of PON1 concentration by fenofibrate. Our findings suggest that the *PON1* Q192R and T-108C polymorphisms modify the therapeutic response of PON1 levels to fenofibrate treatment in these patients.

In this study, microcoated fenofibrate also exerted a beneficial effect on anthropometric parameters as well as blood pressure. It was found that 12 weeks treatment with fenofibrate could significantly reduce both SBP and DBP in our study. Koh *et al*. reported that fenofibrate improved vasomotor function by increasing the percent flow-mediated dilation (228). The increased expression of endothelial nitric oxide synthase activity and diminished thrombin and oxLDL-induced expression of endothelin-1 could also contribute to the beneficial effects of fenofibrate on the improvement in endothelial function (230).

All adverse events experienced during microcoated fenofibrate therapy were transient and no serious adverse events were observed. Adverse events reported in our study were similar to those reported in the other studies which showed that microcoated fenofibrate was generally well tolerated (193, 194). Neither of the patients with transaminases ≥ 3 times ULN nor CK ≥ 5 times ULN were observed.

Lipid lowering therapy with microcoated fenofibrate not only reduced TC, TG, non-HDL-C, oxLDL and apo B levels and increased HDL-C and apo AI levels but also increased PON1 concentration and activity. Decreasing oxLDL and increasing PON1 levels by fenofibrate may be regarded as an additional mechanism of its favourable action and play a critical role in improving oxidative stress and antioxidant capacity.

The understanding of the effects of lipid-lowering drugs on non-lipid risk factors of atherosclerosis is helpful for selecting an optimal treatment according to risk profile of an individual patient. This knowledge is also essential for the development

of more effective therapy. However, there is still no strong clinical evidence that modification of non-lipid risk factors such as PON1 levels, is associated with a reduction of clinical events.

CHAPTER VI
CONCLUSION

Part 1: The study of PON1 status in healthy Thai population

Serum PON1 concentration negatively correlated with WHR, SBP, and DBP, whereas the positive correlation was observed between either HDL-C or apo AI and serum PON1 activity towards paraoxon.

In healthy Thai subjects, genotype and allele frequencies for the *PON1* L55M, Q192R, T-108C and G-909C polymorphisms were 89.55%LL, 10.45%LM (L=0.95, M=0.05), 50.0%QQ, 41.79%QR, 8.21%RR (Q=0.71, R=0.29), 55.22%TT, 41.05%CT, 3.73%CC (T=0.76, C=0.24), 55.22%CC, 34.33%CG, and 10.45%GG (C=0.72, G=0.28), respectively. Linkage disequilibrium between Q192R and L55M, Q192R and T-108C, and Q192R and G-909C was observed.

The *PON1* L55M polymorphism was associated with significant in variation in serum PON1 concentration. The PON1 activity towards paraoxon and diazoxon were significantly influenced by the *PON1* Q192R and G-909C polymorphisms. The *PON1* T-108C polymorphism did not significantly affect neither the variation in serum PON1 concentration nor activity towards any substrates. None of *PON1* polymorphisms significantly affected the variation in PON1 activity towards phenylacetate.

Paraoxon and diazoxon appeared to be discriminating substrates while phenylacetate appeared to be a non-discriminating substrate with respect to the *PON1* polymorphisms.

Stepwise multiple regression analysis indicated that the *PON1* L55M polymorphism was identified as a significant contributor to PON1 concentration. The significant contributors for PON1 activity towards paraoxon were the *PON1* Q192R polymorphism and PON1 concentration. While both *PON1* G-909C and Q192R polymorphisms contributed to the variation in PON1 activity towards diazoxon.

Our data demonstrate that the *PON1* polymorphisms in the healthy Thai population associated with significant differences in serum PON1 concentration and activity as well as lipid parameters. The influence of the *PON1* polymorphisms on the variation in serum PON1 activity is substrate-dependent.

Additionally, not only do allele frequencies of the *PON1* polymorphisms vary between different ethnic populations, but also the linkage disequilibrium patterns between the *PON1* polymorphisms differ among ethnic populations. This study thus firmly establishes a genetic basis for the variation in serum PON1 concentration and activity. Finally, our study provides the first baseline data of PON1 status in the Thai population, which differs significantly from other oriental populations.

Part 2: The study of effect of fenofibrate therapy on PON1 status in patients with low HDL-C levels

Microcoated fenofibrate was effective in lowering TC, TG, non-HDL-C, oxLDL and apo B, and increasing HDL-C and apo AI levels. While, the mean LDL-C was not significantly changed by microcoated fenofibrate therapy. After 6 and 12 weeks of treatment, 47.5% and 55.2% of patients with low HDL-C levels achieved HDL-C treatment goal, HDL-C levels ≥ 40 mg/dL, respectively.

The change of HDL-C and apo AI after treatment negatively correlated with the change of TG, non-HDL-C and oxLDL.

The effect of microcoated fenofibrate on the TG reduction was dependent of the baseline TG levels. The greater baseline of TG level, the greater reduction was achieved.

There were no significant differences in the mean hs-CRP after therapy when compared with those at baseline. Nevertheless, in the subgroup with baseline hs-CRP levels > 3 mg/L, microcoated fenofibrate significantly reduced the mean hs-CRP after 12 weeks of treatment and 53.9% of patients in this subgroup achieved hs-CRP level of < 3 mg/L after treatment.

Microcoated fenofibrate exerted favorable effect on increasing both serum PON1 concentration and activity towards three substrates including paraoxon, phenylacetate and diazoxon. The increased PON1 concentration and activity after microcoated fenofibrate were positively associated with the change of HDL-C and apo

AI and negatively associated with the change of TG. The mechanisms of action of microcoated fenofibrate on increasing PON1 activity could be due to increase in PON1 concentration by induction of *PON1* gene expression and / or the increase in HDL-C and apo AI concentrations since PON1 exists in serum exclusively as a component of HDL.

In patients with low HDL-C levels, genotype and allele frequencies for the *PON1* L55M, Q192R, T-108C and G-909C polymorphisms were 91.67%LL, 8.33%LM (L=0.96, M=0.04), 40%QQ, 50%QR, 10%RR (Q=0.65, R=0.35), 21.67%TT, 40%CT, 38.33%CC (T=0.42, C=0.58), 31.67%CC, 40%CG, and 28.33%GG (C=0.52, G=0.48), respectively.

The *PON1* T-108C polymorphism was associated with significant in variation in serum PON1 concentration. The PON1 activity towards paraoxon and phenylacetate were significantly influenced by the *PON1* Q192R polymorphism. Meanwhile, the significant variation in PON1 activity towards diazoxon was affected by the *PON1* G-909C polymorphism.

The *PON1* Q192R and T-108C polymorphisms influenced on the therapeutic response of PON1 levels to microcoated fenofibrate therapy.

Treatment with microcoated fenofibrate also had beneficial effects on anthropometric parameters as well as blood pressure.

Microcoated fenofibrate was well tolerated and showed the safety profile in Thai patients.

Decreasing oxLDL and increasing PON1 levels by microcoated fenofibrate may be regarded as an additional mechanism of action of its favorable action and play an important role in improving oxidative stress and antioxidant capacity.

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APPENDIX

APPENDIX A

DOCUMENTARY PROOF OF ETHICAL CLEARANCE

Documentary Proof of Ethical Clearance
Research Ethical Committee
Bhumibol Adulyadej Hospital, Royal Thai Air Force
Phaholyothin Road, Bangkhen
Bangkok 10220, Thailand

Title of Project : Paraoxonase1 status in healthy Thai population and effect of fenofibrate therapy on paraoxonase1 status in patients with low high – density lipoprotein cholesterol levels

Principal Investigator : Mrs.Wimon Phuntuwate, M.Sc. (Pharmacology)

Name of Institution: Faculty of Pharmacy, Department of Pharmacology
Mahidol University

Type of approval/acceptance : 1.Clinical Trial Protocol
2. Patient Informed Consent Form : Thai version

Approved by Research Ethical Committee on: 7 October 2002

Gp.Capt. ..... M.D.
(Group Captain Yongyudh Vonglertvidhya)
Chairman Ethical Committee

AVM. ..... M.D.
(Air Vice Marshal Peerapan Prateeprat)
Hospital Director

Date of Approval : 7 October 2002

เอกสารรับรองโครงการวิจัย
โดย
คณะกรรมการจริยธรรมการวิจัย โรงพยาบาลภูมิพลอดุลยเดช
กรมแพทย์ทหารอากาศ กองบัญชาการสนับสนุนทหารอากาศ
ขอรับรองว่า

โครงการ สถานะภาพของเอนไซม์พาราออกซอนเนส1ในประชากรไทยปกติ และผลของการรักษาด้วยยาฟีโนไฟเบรตต่อสถานะภาพของเอนไซม์พาราออกซอนเนส1 ในผู้ป่วยที่มีระดับเอชดีแอลโคเลสเตอรอลต่ำ

โดย นางวิมล พันธุเวทย์

สังกัด นักศึกษาดุษฎีบัณฑิต สาขาเภสัชศาสตรชีวภาพ ภาควิชาเภสัชวิทยา
คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล

เอกสารที่พิจารณา ๑. โครงร่างงานวิจัย
๒. เอกสารข้อมูลสำหรับผู้ป่วยและเอกสารแสดงความยินยอมเข้าร่วมการศึกษา ฉบับภาษาไทย

คณะกรรมการจริยธรรมการวิจัย โรงพยาบาลภูมิพลอดุลยเดช กรมแพทย์ทหารอากาศ กองบัญชาการสนับสนุนทหารอากาศ ได้พิจารณารายละเอียดโครงร่างงานวิจัย เอกสารข้อมูลสำหรับผู้ป่วย เอกสารแสดงความยินยอมเข้าร่วมการวิจัยฉบับภาษาไทย แล้วเห็นว่าไม่ขัดต่อสวัสดิภาพ หรือก่อให้เกิดภยันตรายแก่ผู้ถูกวิจัยแต่ประการใด

จึงเห็นสมควรให้ดำเนินการวิจัยในขอบข่ายของโครงการที่เสนอได้ ณ วันที่ ๗ ตุลาคม ๒๕๔๕

นาวาอากาศเอก.....
(นายแพทย์ยงยุทธ วงศ์เลิศวิทย์)
ประธานคณะกรรมการจริยธรรมการวิจัย

พลอากาศตรี.....
(นายแพทย์พีระพันธ์ ประทีปรัตน์)
ผู้อำนวยการโรงพยาบาลภูมิพลอดุลยเดช
กรมแพทย์ทหารอากาศ กองบัญชาการสนับสนุนทหารอากาศ

No. 137/2004

Documentary Proof of Ethical Clearance
The Committee on Human Rights Related to
Human Experimentation
Mahidol University, Bangkok

Title of Project: Paraoxonase 1 Status in Healthy Thai Population and Effect of Fenofibrate Therapy on Paraoxonase 1 Status in Patients with Low High-Density Lipoprotein Cholesterol Levels
(Thesis for Ph.D.)

Principle Investigator: Mrs. Wimon Phuntuwate

Name of Institution: Faculty of Pharmacy

Approved by the Committee on Human Rights Related to Human Experimentation

Signature of Chairman: .....
(Professor Dr.Srisin Khusmith)

Signature of Head of the Institute: .....
(Professor Dr.Pornchai Matangkasombut)

Date of Approval: 19 NOV 2004

APPENDIX B
INFORMED CONSENT

ใบยินยอมให้ทำการวิจัยในมนุษย์

การวิจัยเรื่อง สถานะภาพของเอนไซม์พาราออกซอนเนส1 ในประชากรไทยปกติ

วันให้คำยินยอมวันที่…...…เดือน……..……..……พ.ศ. ……

ชื่อ……..นามสกุล……..

ที่อยู่……

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัยหรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบัง ซ่อนเร้น จนข้าพเจ้าพอใจ ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าในโครงการวิจัยนี้เมื่อใดก็ได้ และเข้าร่วมโครงการวิจัยนี้โดยสมัครใจและการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปแบบที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้อง กระทำได้เฉพาะกรณี จำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ผู้วิจัยรับรองว่าหากเกิดอันตรายใดๆ อันเนื่องจากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่าตามมาตรฐานวิชาชีพ และจะได้รับการชดเชยรายได้ที่สูญเสียไประหว่างการรักษาพยาบาลดังกล่าว ตลอดจนเงินทดแทนความพิการที่อาจเกิดขึ้น

ผู้วิจัยรับรองว่าหากข้อมูลเพิ่มเติมที่ส่งผลกระทบต่อการวิจัย ข้าพเจ้าจะได้รับการแจ้งให้ทราบโดยไม่ปิดบัง ซ่อนเร้น

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม ..... ผู้ยินยอม

ลงนาม ..... พยาน

ลงนาม ..... พยาน

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจแล้ว และข้าพเจ้าจึงลงนามหรือประทับลายนิ้วหัวแม่มือขวาของข้าพเจ้าในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม ..... ผู้ยินยอม

ลงนาม ..... พยาน

ลงนาม ..... พยาน

ใบยินยอมให้ทำการวิจัยในมนุษย์

การวิจัยเรื่อง ผลของการรักษาด้วยยาฟีโนไฟเบรตต่อสถานะภาพของเอนไซม์พาราออกซอนเนส 1 ในผู้ป่วยที่มีระดับเอชดีแอลโคเลสเตอรอลต่ำ

วันให้คำยินยอม วันที่…..เดือน……..……..……พ.ศ. ……..

ชื่อ…….นามสกุล……..

ที่อยู่……

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัยหรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบัง ซ่อนเร้น จนข้าพเจ้าพอใจ ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าในโครงการวิจัยนี้เมื่อใดก็ได้ และเข้าร่วมโครงการวิจัยนี้โดยสมัครใจและการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปแบบที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้อง กระทำได้เฉพาะกรณี จำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ผู้วิจัยรับรองว่าหากเกิดอันตรายใดๆ อันเนื่องจากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่าตามมาตรฐานวิชาชีพ และจะได้รับการชดเชยรายได้ที่สูญเสียไประหว่างการรักษาพยาบาลดังกล่าว ตลอดจนเงินทดแทนความพิการที่อาจเกิดขึ้น

ผู้วิจัยรับรองว่าหากข้อมูลเพิ่มเติมที่ส่งผลกระทบต่อการวิจัย ข้าพเจ้าจะได้รับการแจ้งให้ทราบโดยไม่ปิดบัง ซ่อนเร้น

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม ..... ผู้ยินยอม

ลงนาม ..... พยาน

ลงนาม ..... พยาน

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจแล้ว และข้าพเจ้าจึงลงนามหรือประทับลายนิ้วหัวแม่มือขวาของข้าพเจ้าในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม ..... ผู้ยินยอม

ลงนาม ..... พยาน

ลงนาม ..... พยาน

APPENDIX C
QUESTIONNAIRE

แบบสอบถาม

ชื่อ……HN……CODE……

ที่อยู่……

1. เพศ

☐ 1. ชาย ☐ 2. หญิง

2. อายุ……..…ปี

☐ 1. น้อยกว่า 21 ปี ☐ 2. 21-30 ปี

☐ 3. 31-40 ปี ☐ 4. 41-50 ปี

☐ 5. 51-60 ปี ☐ 6. 61-70 ปี

3. สัญชาติ

☐ 1. ไทย ☐ 2. อื่นๆ……..

4. เชื้อชาติ

☐ 1. ไทย ☐ 2. อื่นๆ……..

5. สถานภาพสมรส

☐ 1. โสด ☐ 2. สมรส

☐ 3. หย่า/ ม่าย ☐ 4. แยกกันอยู่

6. การศึกษา

☐ 1. ประถมศึกษา ☐ 2. มัธยมศึกษาตอนต้น

☐ 3. มัธยมศึกษาตอนปลาย/ปวช. ☐ 4. อนุปริญญา/ปวส.

☐ 5. ปริญญาตรี ☐ 6. สูงกว่าปริญญาตรี

7. อาชีพ

☐ 1. นักเรียน/นักศึกษา ☐ 2. รับราชการ

☐ 3. รัฐวิสาหกิจ ☐ 4. เกษตรกรรม

☐ 5. ค้าขาย ☐ 6. รับจ้าง

☐ 7. อื่นๆ……

8. ท่านมีโรคประจำตัว หรือไม่

☐ 0. ไม่มี ☐ 1. มี (ระบุโรค)

9. ขณะนี้ท่านมีการรับประทานยาหรือใช้ยาใดเป็นประจำ หรือไม่

(เช่น ฮอร์โมน วิตามินเอ ซี หรืออี)

☐ 0. ไม่มี ☐ 1. มี (ระบุชื่อยา)……..

10. ท่านเคยมีอาการผิดปกติหรือบาดเจ็บ หรือไม่ (ภายใน 1 เดือนที่ผ่านมา)

☐ 0. ไม่มี ☐ 1. มี (ระบุอาการ)……..

11. ประวัติมารดา มีโรคประจำตัว หรือไม่ (เช่น โรคเบาหวาน โรคความดันโลหิตสูง โรคหัวใจ)

☐ 0. ไม่มี ☐ 1. มี (ระบุโรค) ……..

12. ประวัติบิดา มีโรคประจำตัว หรือไม่ (เช่น โรคเบาหวาน โรคความดันโลหิตสูง โรคหัวใจ)

☐ 0. ไม่มี ☐ 1. มี (ระบุโรค) ……..

13. ท่านสูบบุหรี่ หรือไม่ (ภายใน 1 เดือนที่ผ่านมา)

☐ 0. ไม่สูบ

☐ 1. สูบและปัจจุบันยังสูบอยู่ สูบโดยเฉลี่ย…….มวนต่อวัน สูบมานาน……..ปี

☐ 2. เคยสูบ ปัจจุบันหยุดสูบแล้ว เคยสูบโดยเฉลี่ย….มวนต่อวัน หยุดสูบมานาน…...

14. ท่านดื่มเครื่องดื่มที่มีแอลกอฮอล์ หรือไม่ (ภายใน 1 สัปดาห์ที่ผ่านมา)

☐ ไม่ดื่ม ☐ ดื่ม

15. ท่านออกกำลังกาย หรือไม่

☐ 0. ไม่ออกกำลังกาย (ถ้าเลือกข้อนี้ ข้ามไปกรอกข้อ 16)

☐ 1. ออกกำลังกาย (ถ้าเลือกข้อนี้ กรอกข้อ 15.1-15.3)

15.1 ลักษณะของการออกกำลังกาย……

15.2 ระยะเวลาของการออกกำลังกายในแต่ละครั้งนาน

☐ 1. น้อยกว่า 30 นาที

☐ 2. 30-60 นาที

☐ 3. มากกว่า 1 ชั่วโมง

15.3 ความถี่ของการออกกำลังกายใน 1 สัปดาห์

☐ 1. น้อยกว่า 3 ครั้ง

☐ 2. 3-6 ครั้ง

☐ 3. ทุกวัน

16. ท่านทานมังสวิรัติ หรือไม่

☐ 0. ไม่ทาน ☐ 1. ทาน

APPENDIX D
MANUAL FOR DIETARY CONTROL AND EXERCISE

คู่มือคำแนะนำการบริโภคอาหารและการออกกำลังกาย

การควบคุมอาหาร

1. โดยการลดปริมาณไขมันที่รับประทานให้น้อยลง
2. โดยการลดปริมาณกรดไขมันอิ่มตัว โดยหลีกเลี่ยงอาหารที่มีกรดไขมันอิ่มตัวสูง เช่น เนื้อสัตว์ติดมัน มันสัตว์ต่างๆ และน้ำมันพืชบางชนิด เช่น น้ำมันมะพร้าว น้ำมันปาล์ม และกะทิ นม และผลิตภัณฑ์นมต่างๆ (เนย, เนยแข็ง, ครีม ฯลฯ) ควรรับประทานกรดไขมันไม่อิ่มตัวมากขึ้น
3. โดยกรดไขมันไม่อิ่มตัวชนิดเชิงเดี่ยวสามารถพบในน้ำมันมะกอก ส่วนกรดไขมันไม่อิ่มตัวชนิดเชิงซ้อนซึ่งสามารถพบในน้ำมันถั่วเหลือง น้ำมันข้าวโพด น้ำมันรำข้าว น้ำมันงา น้ำมันเมล็ดดอกทานตะวัน น้ำมันเมล็ดฝ้าย และปลาทะเลชนิดต่างๆ เช่น ปลาทูน่า ปลาซาลมอน ปลาเทร้าต์ และปลาซาดีน เป็นต้น เนื่องจากมีกรดไลโนเลอิกมาก สามารถลดโคเลสเตอรอล และไตรกลีเซอร์ไรด์ในเลือดได้
4. ควรรับประทานอาหารที่มีโคเลสเตอรอลสูงให้น้อยลง โดยหลีกเลี่ยงอาหารที่มีโคเลสเตอรอลสูง เช่น ไข่แดง เครื่องในสัตว์ ปลาหมึก กุ้ง หนังสัตว์ มันสัตว์
5. ควรรับประทานอาหารที่มีเส้นใยให้มากขึ้น เช่น ผัก ผลไม้ ธัญญพืชต่างๆ พร้อมทั้งลดอาหารประเภทแป้งและน้ำตาล ซึ่งจะช่วยลดไขมันในเลือดได้ด้วย ทั้งนี้เนื่องมาจากร่างกายสามารถ

สร้างไตรกลีเซอร์ไรด์ และโคเลสเตอรอลได้จากอาหารประเภทนี้ ควรหลีกเลี่ยงการผัดผักด้วยน้ำมันมากๆ และหลีกเลี่ยงผลไม้ที่มีรสหวานมาก

6. ควรรับประทานอาหารที่มีโปรตีนอย่างเหมาะสม ได้แก่ เนื้อสัตว์ที่ไม่ติดมัน ปลาต่างๆ นมพร่องไขมัน ถั่วเมล็ดแห้ง หรือเต้าหู้ทุกชนิดที่ไม่ผสมไข่ งดเครื่องในสัตว์ทุกชนิด หนังสัตว์ ส่วนอาหารทะเลรับประทานได้สัปดาห์ละ 1 ครั้ง ไข่พยายามหลีกเลี่ยงการรับประทานไข่แดง รับประทานเฉพาะไข่ขาว งดไข่ปูและไข่ปลาด้วย ควรเลือกดื่มนมพร่องไขมันชนิดจืด
7. ควรรับประทานอาหารเช้า 25% อาหารกลางวัน 50% และอาหารเย็น 25% ของพลังงานทั้งหมดที่รับประทานต่อวัน
8. การรับประทานอาหารในแต่ละมื้อ ควรรับประทานอาหารอย่างช้าๆ เคี้ยวให้ละเอียด เพราะกว่าร่างกายจะรู้สึกอิ่ม จะต้องใช้เวลาประมาณ 20 นาที ฉะนั้นถ้ารีบรับประทานอาหาร จะทำให้รับประทานอาหารได้มากภายใน 20 นาที

การออกกำลังกาย

การออกกำลังกายจะเป็นการเพิ่มการใช้ไขมันในร่างกายทำให้โคเลสเตอรอลลดลง เพิ่มเอชดีแอลโคเลสเตอรอล เพิ่มสมรรถภาพการทำงานของหัวใจ และยังเพิ่มความแข็งแรงของร่างกาย

การออกกำลังกายที่เหมาะสมในผู้ป่วย ได้แก่

- การเดินติดต่อกันนาน 40-60 นาที อย่างน้อยสัปดาห์ละ 3 ครั้ง จะช่วยเพิ่มการเผาผลาญไขมัน หรือ
- การเดินเร็วติดต่อกันนาน 20-30 นาที อย่างน้อยสัปดาห์ละ 3 ครั้ง

APPENDIX E
PICTURES OF PCR PRODUCTS

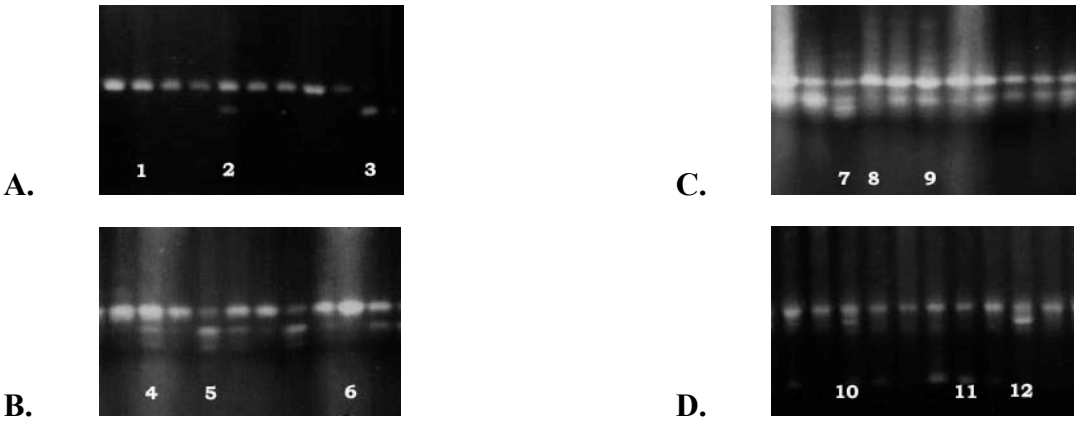

Figure 54. Detection of PON1 DNA amplification by restriction endonuclease using 3% agarose gel with EtBr staining

A. Position 55	Lane 1: PCR product of LL genotype
	Lane 2: PCR product of LM genotype
	Lane 3: PCR product of MM genotype
B. Position 192	Lane 4: PCR product of QR genotype
	Lane 5: PCR product of RR genotype
	Lane 6: PCR product of QQ genotype
C. Position -108	Lane 7: PCR product of CC genotype
	Lane 8: PCR product of TT genotype
	Lane 9: PCR product of CT genotype
D. Position -909	Lane 10: PCR product of CG genotype
	Lane 11: PCR product of CC genotype
	Lane 12: PCR product of GG genotype

BIOGRAPHY

NAME	Mrs. Wimon Phuntuwate
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