University of Montreal

Regulation of macrophage lipoprotein lipase expression by peroxisome proliferator-activated receptor agonists

By

Ling Li

Department of Biomedical Sciences

Faculty of medicine

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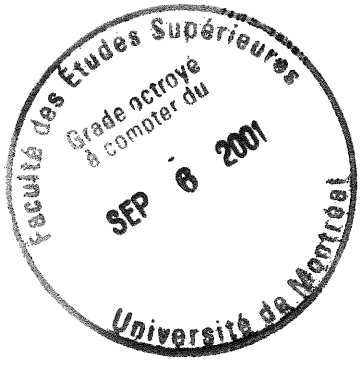

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This thesis entitled

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Presented by

Ling Li

Has been evaluated by a jury made up of the following people

President of jury :	Dr. Jane Aimée Montgomery
Director of research :	Dr. Geneviève Renier
Member of jury :	Dr. Jeffrey Stuart Cohn

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Thesis accepted on :

RÉSUMÉ

L'athérosclérose est une complication importante du diabète de type 2. La lipoprotéine lipase (LPL), une enzyme produite par les macrophages dans la paroi artérielle favorise le développement de l'athéroclérose et est surexprimée dans le diabète humain. Les *peroxisome proliferator-activated receptors* (PPARs) sont des facteurs de transcription qui médient divers effets pléiotropiques incluant la régulation de gènes impliqués dans le métabolisme lipidique tels le gène de la LPL et ceux contrôlant l'inflammation. Dans la présente étude, nous avons déterminé l'effet des agonistes de PPAR alpha (α) et gamma (γ) sur l'expression de la LPL macrophagique. Nos résultats démontrent que l'incubation des cellules THP-1 et des macrophages dérivés de monocytes humains en présence de ligands de PPARα et PPARγ augmentent l'expression du gène de la LPL, sa masse immunoreactive, et son activité de manière dose- et temps-dépendants. Les ligands de PPAR semblent moduler l'expression du gène de la LPL en augmentent la liaison des PPARs à l'élément de réponse des PPARs (PPRE) situé dans le promoteur du gène de la LPL. Ces agonistes agissent également à un niveau posttranscriptionel. En effet, ils régulent l'expression génique de la LPL en augmentant la demi-vie de son ARNm dans les cellules THP-1. La présente étude démontre pour la première fois un effet direct des agonistes de PPAR sur l'expression génique et la sécrétion de la LPL macrophagique. Une compréhension détaillée du rôle des PPARs dans la régulation de la biologie vasculaire et le développement de nouveaux ligands de PPAR dissociant les propriétés pro- et anti-athérogéniques de ces agents offrira de nouvelles stratégies dans le traitement de l'athérosclérose.

SUMMARY

Atherosclerosis is a major complication of type 2 diabetes. Lipoprotein lipase (LPL), an enzyme produced by macrophages in the arterial wall favors the development of atheroclerosis and is upregulated in human diabetes. Peroxisome proliferator-activated receptors (PPARs) are transcription factors which mediate pleiotropic effects including regulation of genes involved in lipid metabolism such as LPL and control of inflammation. In the present study, we determined the effect of PPAR alpha (α) and gamma (γ) agonists on macrophage LPL control. Our results show that incubation of THP-1 and human monocyte-derived macrophages in the presence of PPARα or PPARγ ligands enhance LPL gene expression, immunoreactive mass, and activity in a dose-, time-, and ligand affinity-dependent manner. PPAR ligands appear to modulate macrophage LPL gene expression through PPAR binding to a specific response element termed peroxisome proliferator responsive element (PPRE) in the promoter of the LPL gene. These compounds also act at the posttranscriptional level to regulate macrophage LPL mRNA expression as reflected by the slower rate of decay of LPL mRNA in THP-1 cells exposed to PPAR α and γ ligands. The present study provides the first evidence of a direct regulatory effect of PPAR agonists on human macrophage LPL gene expression and secretion. A detailed understanding of the role of PPARs in the regulation of vascular biology and the development of novel PPAR ligands that dissociate pro- and anti-atherogenic properties will offer new strategies in the treatment of atherosclerosis.

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LIST OF ABREVIATIONS

AA : arachidonic acid

ACS : acyl-CoA synthetase

AGEs : advanced glycation end products

AP-1, -2 : activated protein –1 and 2

ApoCII : apolipoprotein CII

BRL49653 : rosiglitazone

cAMP : cyclic adenosine monophosphate

CHD : coronary heart disease

COX-2 : cyclo-oxygenase 2

C. pneumoniae : *Chlamydia pneumoniae*

CPT-1 : carnitine palmitoyltransferase

CS : cystathionine synthase

DBD : DNA-binding domain

EPA : eicosapentaenoic acid

FAT : fatty acid translocase

FATP : fatty acid transport protein

FFA : free fatty acids

FGF-2 : fibroblast growth factor –2

FSE : fat specific element

GH : growth hormone

GRE : glucocorticoid responsive element

HDL : high-density lipoprotein

HETE : hydroxyeicosatetraenoic acid

HF-3 : hepatic factor-3

HODE : hydroxyoctadecenoic acid

H. pylori : *Helicobacter pylori*

HSPG : heparan sulfate proteoglycans

ICAM-1 : intercellular adhesion molecule-1

IFN-γ : interferon-γ

IL-1 : interleukin-1

IL-6 : interleukin-6

IL-8 : interleukin-8

IL-12 : interleukin-12

iNOS : inducible NO synthase

LA : linoleic acid

LDL : low-density lipoproteins

LFA-1 : lymphocyte function-related antigen-1

LIF : leukemia inhibitory factor

LP : lipoprotein

Lp(a) : lipoprotein (a)

Lp(β) : lipoprotein (β)

LPL : lipoprotein lipase

LPS : lipopolysaccharide

LTB4 : leukotriene B4

MAPK : mitogen-activated protein kinase

MCP-1 : monocyte chemotactic protein-1

M-CSF : macrophage-colony stimulating factor

MMP : matrix metalloproteinases

MTHFR : methylenetetrahydrofolate reductase

NF-κB : "nuclear factor-kappa B"

NO : nitric oxide

NOS : nitric oxide synthase

OA : oleic acid

Oct-1 : octamer-1

$O_2^{-}\bullet$: superoxide anion

oxLDL : oxidized low-density lipoprotein

PA : palmitic acid

PAI-1 : plasminogen activator inhibitor-1

PDGF : platelet-derived growth factor

PGs : prostaglandins

PKC : protein kinase C

PLC : phospholipase C

PPAR : peroxisome proliferator-activated receptors

PPRE : PPAR response element

RAS : renin-angiotensin system

ROS : reactive oxygen species

RXR : retinoic acid receptors

SA : stearic acid

SMCs : smooth muscle cells

sPLA2 : secreted phospholipase A2

SP1 : stimulatory protein1

TGF-β : transforming growth factor β

TG : triglycerides

TNF-α : tumor necrosis factor α

TZDs : thiazolidinediones

TRE : thyroid responsive element

Type II sPLA2 : type II-secreted phospholipase

VCAM-1 : vascular cell adhesion molecule-1

VEGF : vascular endothelial growth factor

VLDL : very low-density lipoproteins

ACKNOWLEDGEMENTS

First and foremost, I would like to express my most sincere appreciation to my supervisor Dr. Geneviève Renier, for her solid and skillful guidance, as well as her encouragement throughout this dissertation. I feel honoured to have been her student, and value greatly the wisdom she has shared with me. Furthermore, I acknowledge two of my colleagues, Marie-Claude Beauchamp and Dr. Jean-Claude Mamputu, both enthusiastic supporters of my efforts . Finally I would like to honor my great country China, that gave me this precious opportunity to explore personal enrichment, and to pursue my professional passion of medical research .

DEDICATION

I dedicate my work to my loving and caring parents, who have always been there to encourage and support me. You both taught me the importance of friendship, respect, and love, for all aspects of life. I dedicate my work to my husband and lovely daughter, whose love and strength have sustained my energy to complete this work. In addition, I dedicate this work to my brother, as well as my best friends, who through their goodness of nature have given me, as the song goes, "the wind beneath my wings."

I. INTRODUCTION

I. Introduction

I.I. Atherosclerosis

I.I.1. General

Atherosclerosis is a chronic, multifactorial and progressive disease characterized by the accumulation of lipids and fibrous elements in the large and medium arteries. Epidemiological studies over the past 50 years have identified numerous risk factors for atherosclerosis. These can be grouped into factors with an important genetic component and those that are largely environmental. Among the primary events in atherosclerosis are the accumulation of low density lipoproteins (LDL) in the subendothelial space [Guyton et al. 1996, Van De Graff et al. 1992] and the adhesion of inflammatory cells, namely monocytes and T-lymphocytes, to the endothelium. The notion that atherosclerosis is an immune-mediated inflammatory disease is now widely accepted and provides the basis for the development of new strategies in the diagnosis and management of this disease.

I.I.2. Development and progression of the atherosclerotic lesion

I.I.2.1. Endothelial dysfunction in atherosclerosis

The vascular endothelium is considered to be a monolayer layer acting as a selectively permeable barrier between blood and tissues. It is a dynamic and heterogeneous organ. It has secretory, synthetic, metabolic, and immunological functions and can generate effector molecules, such as nitric oxide (NO),

prostacyclin, platelet-derived growth factor (PDGF), angiotensin II, and endothelin [Gotlieb et al. 1996] that regulate thrombosis, inflammation, vascular tone and vascular remodeling. The endothelium has important anti-coagulant and fibrinolytic functions. Endothelial dysfunction is closely linked to the occurrence of vascular diseases and preceeds the development of atherosclerosis. It is characterized by major functional alterations, including decreased vasodilatory response, increased vascular permeability and enhanced pro-thrombotic and pro-coagulant activities. Increased endothelial permeability is considered as one of the earliest events in atherogenesis. Increased permeability of endothelium to macromolecules such as LDL and enhanced migration of inflammatory cells to the subintimal space through the effect of oxidized LDL (oxLDL) [Rajavashisth et al. 1990], monocyte chemotactic protein 1 (MCP-1), interleukin-8 (IL-8), PDGF, macrophage-colony stimulating factor (M-CSF), and osteopontin [Giachelli et al. 1998] **(Figure 1, A and B)** result in the formation of early atherosclerotic lesions or fatty streaks.

I.I.2.2. Fatty-streak lesion

As mentioned above, early atherosclerotic lesions, or fatty streaks are characterized by the accumulation of LDL and the migration of monocytes and lymphocytes into the subendothelial space of the arterial wall. Fatty streaks initially consist of lipid-laden monocytes and macrophages (foam cells) mixed together with variable numbers of T lymphocytes [Ross 1999]. Rosenfeld et al. [1990] found that the formation of foam cells is mediated mainly by M-CSF, tumor

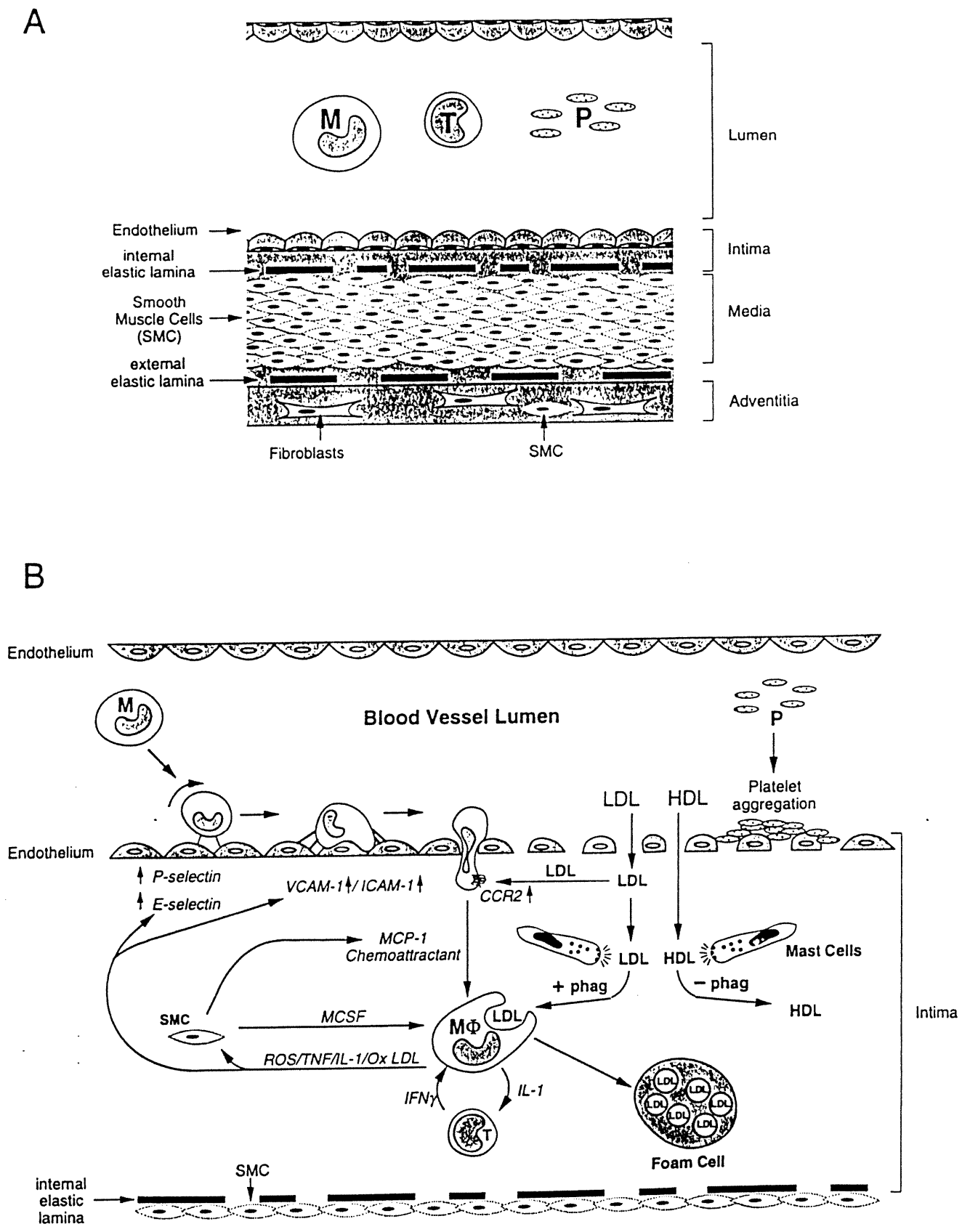


Fig. 1. Endothelial dysfunction and fatty streak formation [Sullivan et al 2000].

necrosis factor α (TNF-α), and interleukin-1(IL-1). Later these cells are joined by various numbers of smooth muscle cells (SMCs). SMC migration is stimulated by PDGF, fibroblast growth factor–2 (FGF-2) and transforming growth factor β (TGF-β) [Boring et al. 1997, Boisvert et al. 1998]. Platelet adherence and aggregation are also observed and are stimulated by integrins, P-selectin, fibrin, thromboxane A_2, and tissue factor [Ross 1999] **(Figure 1)**.

I.I.2.3. Advanced, complicated lesion of atherosclerosis

As fatty streaks progress to intermediate and advanced lesions, they tend to form a fibrous cap that walls off the lesion from the lumen. This represents a type of healing or fibrous response to the injury. This fibrous cap covers a necrotic core containing leukocytes, lipid, and debris [Ross 1999].

I.I.2.4. Unstable fibrous plaques in atherosclerosis

Macrophages release metalloproteinases and other proteolytic enzymes at sites of thinning of the fibrous cap. These enzymes cause degradation of the matrix, which leads to hemorrhage from the vasa vasorum or from the arterial lumen, resulting in thrombus formation and arterial occlusion [Ross 1999]. Recent studies have shown that activated T cells stimulate metalloproteinase production by macrophages in the lesions promoting plaque instability [Schönbeck et al. 1997]. Erosion and rupture of the fibrous cap often occur at the shoulders of the lesion, where macrophages preferentially accumulate [Davies

1990]. Plaque rupture and thrombosis are notable complications of advanced lesions that lead to unstable coronary syndromes or myocardial infarctions.

I.I.3. Risk factors

I.I.3.1. Hypercholesterolemia and dyslipoproteinemia

High plasma levels of cholesterol and pro-atherogenic lipoproteins represent major risk factors for cardiovascular diseases. In particular, increased levels of circulating LDL is considered as a major risk factor of coronary heart disease and one of the earliest events in atherogenesis is the accumulation of LDL in the vessel wall. Retention and modification of LDL are key factors in lesion formation. LDL can be modified by several mechanisms, including oxidation, glycation, aggregation, association with proteoglycans, or incorporation into immune complexes [Steinberg et al. 1997, Khoo et al. 1998, Khoo et al. 1992, Navab et al. 1996]. Progressive oxidation of LDL takes place during the process of LDL particle trapping in arteries. OxLDL is internalized by macrophages through surface scavenger receptors [Steinberg et al. 1997, Khoo et al. 1992, Navab et al. 1996, Griendling et al. 1997, Han et al. 1997]. The uptake of oxLDL by macrophages results in intracellular cholesterol ester accumulation and foam cell formation. Macrophage-derived foam cells are present in all stages of atherogenesis and play a key role in the development and progression of atherosclerosis. Lipoprotein (a) (Lp(a)) closely resembles LDL in its content of cholesterol and apolipoprotein B-100 but differs by the presence of an attached glycoprotein, known as apoprotein (a). The involvement of Lp(a) in

the pathogenesis of atherosclerosis was strongly suggested by the presence of Lp(a) in human atherosclerotic lesions [Rath et al. 1989, Jurgen et al. 1993]. Numerous cross-sectional and prospective studies have also revealed associations between high plasma levels of Lp(a) and atherosclerotic vascular diseases, such as coronary heart disease and stroke [Utermann 1995, Djurovic et al. 1997]. The effect of high plasma levels of Lp(a) in development of atherosclerosis has also been well-studied in transgenic animal models [Lawn et al. 1992, Fan et al. 2001]. Although the importance of high LDL cholesterol as a risk factor for coronary artery disease is well established, the role of hypertriglyceridemia as an independent risk factor for the development of vascular diseases is still controversial. Various factors associated with hypertriglyceridemia, including dyslipoproteinemia, alterations of hemostatic processes, obesity and hypertension may account for the deleterous effect of high triglyceride levels on the atherosclerotic process [Malaguarnera et al. 2000]. Although, the meta-analysis of Austin and Hokanson, 1998 has recently suggested that hypertriglyceridemia is an independent risk factor for cardiovascular diseases, many investigators still believe that triglyceride-rich lipoproteins cannot migrate through the vascular endothelium [Faegerman, 1998]. Recently it has been suggested that postprandial hypertriglyceridemia may cause endothelial dysfunction via enhanced oxidative stress [Bae et al. 2001].

I.I.3.2. Hypertension

The renin-angiotensin system (RAS) plays an important role in the pathogenesis of cardiovascular disease. Angiotensin II, the principal product of the RAS, regulates numerous cellular responses. For example, angiotensin binds to specific receptors on SMC, eliciting the activation of phospholipase C (PLC) and leading to increased intracellular calcium concentrations and SMC hypertrophy [Gibbons et al. 1992]. Angiotensin II also decreases NO production, increases oxidative stress [Yanagitani et al. 1999] and induces the activation of NF-kB [Kranzhöfer et al. 1999], protein kinase C (PKC) [Henrion et al. 1996] and mitogen activated protein kinases (MAPK) [Haendeler and Berk, 2000]. This factor also leads to increased expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [Conzalez et al. 2000] contributing thereby to the accumulation of inflammatory cells in the vessel wall. High angiotensin II levels in hypertensive patients favors atherogenesis by stimulating the growth of SMC [Chobanian et al. 1996].

I.I.3.3. Diabetes

Patients with diabetes are at high risk of cardiovascular diseases [Garcia et al. 1974]. Several risk factors associated with diabetes including dyslipidemia, obesity, hypertension, hyperglycemia and hyperinsulinemia contribute to the accelerated atherosclerosis in human diabetes. Recent studies suggest that hyperglycemia is a major risk factor for the development of diabetic

macrovascular complications (UK Prospective Diabetes Study, 1998). Multiple biochemical alterations including the polyol pathway, advanced glycation end products (AGEs), oxidative stress, PKC activation seem to be involved in the development of diabetic vasculopathies.

I.I.3.4. Homocysteine

Homocysteine is a non-protein-forming sulfhydryl amino acid derived from methionine metabolism. Hyperhomocystinemia is considered to be a novel risk factor for atherosclerotic disease in the coronary, cerebral, and peripheral arterial circulation. Hyperhomocystinemia seen in general adult populations is associated with cystathionine synthase (CS) and methylenetetrahydrofolate reductase (MTHFR) deficiencies [McCully 1996, Mudd et al. 1985, Nehler et al. 1997, Nyard et al. 1997, Malinow 1995]. Mild homocysteine increase occurs in 20-30% of patients with atherosclerotic disease and usually, treatment with folic acid returns plasma homocysteine concentrations to normal [Yarnell 1991]. Ongoing trials are actually assessing the effect of folate treatment on outcomes.

Several mechanisms have been proposed to explain the atherogenic properties of homocysteine. These mechanisms include endothelial dysfunction [Brattström 2000], oxidative damage [Wall et al. 1980, Starkebaum et al. 1986], SMC proliferation [Tsai et al. 1994], activation of the PKC/c-fos signalling pathway [Dalton et al. 1997], platelet aggregation [Durand et al. 1997] and activation of the coagulation pathway [Welch et al. 1997].

I.I.3.5. Coronary artery calcification

Ectopic vascular calcification occurs commonly in atherosclerosis. Its presence and location in coronary arteries have been examined by ultrafast computed tomography scanning, providing interesting new information in patients [Fallavallita et al. 1994, Janowitz 2001]. The recent finding that TNF-α promotes, through the cyclic adenosine monophosphate (cAMP) pathway, osteoblastic differentiation of vascular cells [Tintut et al. 2000] suggests a role of pro-inflammatory cytokines in the vascular calcification process.

I.I.3.6. Family history, age and gender

It is clear that positive family history, advanced age and male gender increase the risk of developing coronary artery disease [Sianiko et al. 1990]. Numerous studies have shown that individuals with a family history of coronary heart disease (CHD) are at higher risk of atherosclerosis. For example, Pankow et al. [1997] investigated family CHD risk scores and found higher traditional CHD risk factors in individuals with a family history of heart disease.

Because of protection by female hormones, the onset of CHD tends to be delayed in women by about 10 years with some catch-up after menopause [Matthews et al. 1989]. This is the rationale for the administration of estrogens to post-menopausal women.

I.I.3.7. Obesity and smoking habit

The relationship between being overweight and cardiovascular disease was a matter of debate for many years. Obesity, especially visceral obesity, is associated with a cluster of metabolic complications increasing the risk of type 2 diabetes and CHD. For example, obese patients with visceral obesity show increased glycemic and insulinemic responses to an oral glucose load compared to normal weight individuals or compared to obese individuals with low visceral adiposity [Kothari et al. 1998]. Viscerally obese patients are also characterized by an unfavourable plasma lipid profile which includes elevated triglyceride and apolipoprotein B concentrations, reduced high density lipoproteins (HDL)-cholesterol levels as well as increased proportion of small, dense LDL particles [Despres et al. 2000].

Despite the epidemiological evidence linking cigarette smoking with cardiovascular disease, the precise components of cigarette smoke responsible for this relationship and the mechanisms by which they exert their effect have not yet been fully elucidated. Cigarette smoke is a complex mixture and only a few components have been extensively studied. Nicotine and carbon monoxide are much less damaging than is whole smoke. There is considerable evidence that cigarette smoking can result in both morphological and biochemical endothelial disturbances both *in vivo* and *in vitro* [Michael Pittilo 2000].

I.I.3.7. Infection

Herpes viruses and *Chlamydia pneumoniae* are two main types of infectious microorganisms that have been shown to correlate with the incidence of atherosclerosis [Libby et al. 1997, Hendrix et al. 1990, Jackson et al. 1997]. Both organisms have been found in atheromatous lesions in coronary arteries [Hendrix et al. 1990, Jackson et al. 1997]. The role of infectious agents in the pathogenesis of atherosclerosis is discussed in the following section.

I.I.4. Pathogenesis of atherosclerosis

I.I.4.1. Endothelial dysfunction hypothesis

As mentioned above, the vascular endothelium is an unicellular layer acting as a semi-permeable membrane between blood and tissues. It has been demonstrated that the endothelium performs a large range of important biological functions, participating in several metabolic and regulatory pathways.

The endothelium retains a reduced vasomotor tone, prevents leukocyte and platelet adhesion, and inhibits the proliferation of vascular SMCs under physiological conditions. Conversely, endothelial dysfunction plays a pathogenic role in the initial development of atherosclerosis [Ross 1993, Choen 1995, Schwartz et al. 1981]. Abundant evidence has proven that endothelial dysfunction preceeds the development of atherosclerotic lesions [McLenachan et al. 1990, Harrison et al. 1987, Fish et al. 1988].

Ludmer et al. [1986] demonstrated that atherosclerotic coronary arteries contracted in response to intracoronary infusion of acetylcholine, whereas normal

coronary arteries became dilated. Later, Celermajer et al. [1992] documented endothelial dysfunction in children and otherwise healthy young adults with risk factors for atherosclerosis.

Besides locally increased antagonists that can reduce endothelium-derived vasodilators, two other factors involved in endothelial dysfunction have been well-studied. NO has been frequently considered to be the major cause of endothelial dysfunction in various clinical conditions. It has been established that NO released from the endothelium is decreased in patients with coronary atherosclerosis [Ludmer et al. 1986, Vita et al. 1989]. Diminished NO production leads to decreased endothelium-dependent vasodilation, increased platelet aggregation and adhesion of monocytes to the endothelium, and enhanced vascular SMC proliferation.

Another mechanism involved in endothelial dysfunction is the elevation of endothelin-1. High plasma endothelin-1 concentrations have been reported in myocardial infarction, cardiogenic shock, unstable angina pectoris, coronary artery disease in general, cardiac failure, and essential hypertension [Cernacek et al. 1989, Shichiri et al. 1990].

I.I.4.2. Lipid infiltration hypothesis

In the early 19th century, von Rokitansky [1852] found that the most characteristic feature of atherosclerosis is cholesterol accumulation in the intima. Much later, the lipid infiltration hypothesis was supported by the studies of Deng et al. [1993] demonstrating the presence of atherosclerotic lesions in rabbits fed

a cholesterol-enriched diet. The lipid infiltration hypothesis states that increased circulating levels of LDL lead to their infiltration in the vessel wall and thereby to the development of atheorsclerosis. This hypothesis has been reinforced over the past years by clinical studies showing a direct correlation between plasma cholesterol levels and CHD and demonstrating a reduction of cardiovascular events by normalization of plasma lipid levels [Castelli et al. 1990, Lipid Research Clinics Program 1984, Brown et al. 1990].

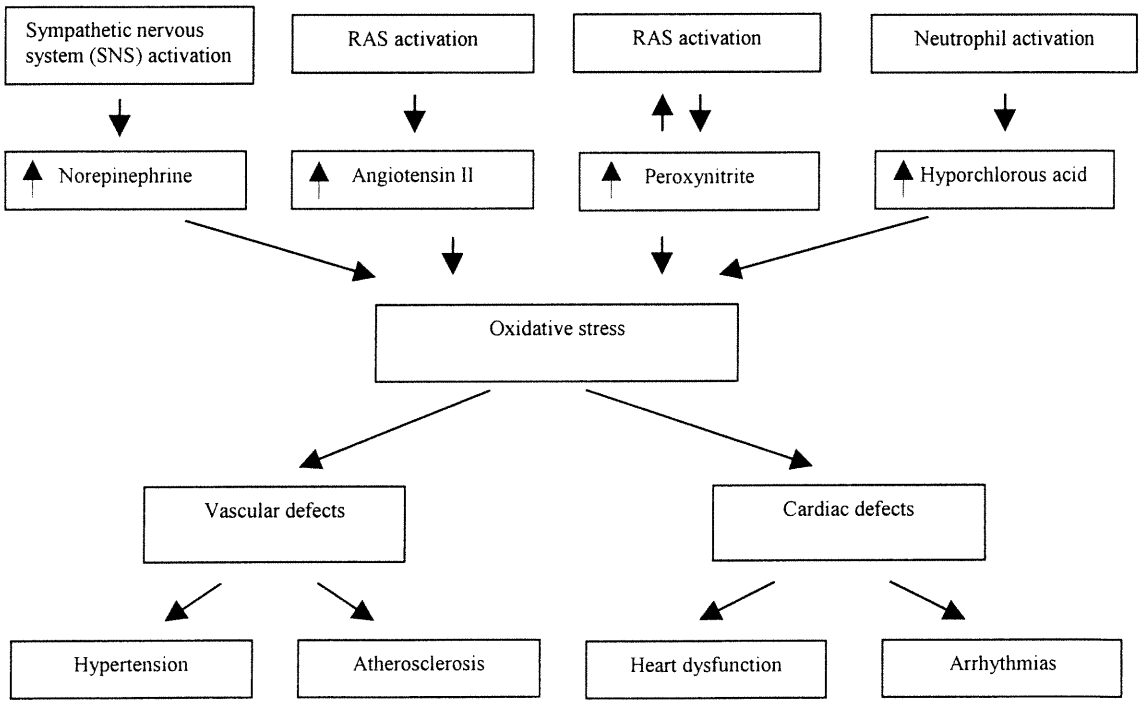
The lipid infiltration theory alone does not explain everything. Indeed, high cholesterol levels and inflammation appear to be two essential components in the pathogenesis of atherosclerosis and both components seem to play a key role in the formation of the atherosclerotic lesion. OxLDL particles are carriers of cholesterol into arterial wall macrophages [Brown et al. 1983, Steinberg et al. 1989], and macrophages in turn become activated by these particles [Wick et al. 1995].

I.I.4.3. Oxidative stress hypothesis

This hypothesis proposes that many pathogenic mechanisms in atherogenesis may result from increased oxidative stress resulting from increased reactive oxygen species (ROS) production. ROS are reactive chemical entities that can be classified into two categories: free radicals, and non-radical derivatives [Kukreja et al. 1994]. ROS have been implicated in the genesis of vascular abnormalities and subsequent cardiovascular tissue injury [Kukreja et al. 1992, 1994]. It has been suggested that ROS may contribute to endothelial

dysfunction in patients with atherosclerosis and that antioxidants such as probucol and ascorbic acid may improve endothelium-dependent relaxation in these subjects [Anderson et al. 1995]. Oxidative inactivation of NO by the superoxide anion (O_2^-) has also been proposed as a plausible explanation for endothelial dysfunction [Harrison 1997]. Finally, oxidation of NO by O_2^- may also play a role in the oxidation of lipoproteins [Beckman et al. 1990].

Because oxLDL plays a key role in atherosclerosis, treatment with antioxidants might be beneficial in preventing or retarding its progression. However, data from randomized intervention trials are still controversial [Aviram 1999] and therefore do not allow for the time being any population-wide recommendations regarding antioxidant supplementation for prevention of cardiovascular disease. A schematic diagram depicting the role of extra-cardiac and extra-vascular systems in the genesis of oxidative stress and development of cardiovascular alterations is presented below. Fig.2 [Dhalla et al. 2000].



I.I.4.4. Hypothesis of infection

There is reasonable evidence suggesting that infection may contribute to the pathogenesis of atherosclerosis. Several studies have addressed the possible role of infectious agents in the development of atherosclerosis. One interesting candidate is *Chlamydia pneumoniae* (*C.pneumoniae*), a human respiratory pathogen, which has been linked in both sero-epidemiological and immunohistochemical studies with CHD [Saikku et al, 1998, Elkind et al, 2000, Schmidt et al, 2000]. Results of animal experiments and preliminary intervention trials with antibiotics [Gurfinkel et al 1999] further suggest a role of this pathogen in atherogenesis. A second infectious agent is *Helicobacter pylori* (*H. pylori*), a pathogen involved in peptic ulcer [Danesh et al. 1997]. *H. pylori* DNA is evident in a considerable number of atherosclerotic plaques, but is absent in healthy vascular walls [Farsak et al. 2000].

These infectious processes could begin in early childhood and only become manifest with aging. An immunological imbalance could develop gradually when defense mechanisms begin to deteriorate as a result of advancing age and accompanying diseases.

I.I.4.5. Hypothesis of inflammation

Atherosclerosis is an inflammatory disease. Several studies have firmly established that immune mechanisms play a key role in the pathogenesis of atherosclerosis. In fact, atherosclerotic lesions can best be described as an

inflammatory disease because they represent a series of highly-specific cellular and molecular responses [Ross et al. 1973, 1986, *Idem* 1976].

Atherosclerosis is a chronic inflammatory response that causes activation of resident macrophages, mast cells, and T lymphocytes with recruitment of leukocytes and their precursors. There is evidence of subpopulations of activated and non-activated macrophages [Galis et al. 1994, Falkenberg et al. 1998] and T cells [de Boer et al. 1997] within plaques. Most studies that relate to inflammation and atherosclerosis focus on macrophages, the obvious reason being that these cells play an important role not only as lipid scavenger cells but also as immunocompetent cells secreting pro-inflammatory cytokines and growth factors. Indeed, activated macrophages are a major source of various molecules, such as PDGF and (interleukin-6) IL-6, which induce SMC migration and proliferation within the neointima of developing plaques [Jang et al. 1993, Hansson et al. 1994]. Macrophages affect endothelial-dependent vasoconstriction/vasodilation via the release of non-oxidative and oxidative products [Marceau 1996]. More than 85% of macrophages within atherosclerotic lesions express the lymphocyte function-related antigen-1 (LFA-1), [Watanabe et al. 1998], a monocyte/macrophage ligand for ICAM-1. This adhesion molecule plays an important role in atherosclerosis lesion formation by inducing monocyte binding to endothelium and monocyte/macrophage retention in the vessel wall.

Lymphocytes are present in each phase of the atherosclerotic process. Although T cells are not as common as macrophages in the atherosclerotic lesion, they could play an important regulatory role in atherogenesis because of

their capacity to control macrophage activity and inflammation. Among the cytokines that resting T cells secrete, interferon-γ (IFN-γ) is the major priming signal for macrophage activation. It leads to the production of macrophage-derived cytokines, such as IL-1 and TNF-α, as well as to the secretion of matrix metalloproteinases (MMP) [Moon et al. 1988. Advanced human plaque, which is dominated by T cells of the Th1 type, i.e. INF-γ-producing $CD4^+$ T cells, is possibly due to the local vascular production of IL-12 [Uyemura et al. 1996]. T cells within atherosclerotic lesions could also react with self-antigens and thus generate an autoimmune response with inflammatory changes characteristic of these lesions. Recently, it was proposed that a significant proportion of plaque $CD4^+$ T cells recognize oxLDL as an $HLA\text{-}D^R$-dependent antigen [Stemme et al. 1995]. This indicates that oxLDL not only activates, and is internalized by, macrophages but is also recognized as a local antigen by immune cells of the plaque.

Recent studies have provided evidence that mast cells are present in various stages of atherosclerotic lesions. Mast cells can assist in the recruitment of monocytes and lymphocytes into vascular tissues, thereby propagating the inflammatory response. Mast cell enzymes might activate pro-metalloproteinases, destabilizing atheromatous plaques via the secretion of pro-inflammatory cytokines. Mast cells can also facilitate foam cell formation by promoting cholesterol accumulation.

Inflammatory cells infiltrate vascular tissues by releasing cytokines and proteases. They also induce ROS and trigger vasoconstriction/vasodilation

[Peitzman et al. 1995], thrombus formation [Lassila et al. 1993, Nielsen et al. 1998], neointimal growth [Hansson et al. 1994, Wilensky et al. 1995], angiogenesis, and tissue remodeling [Wilensky et al. 1995, Mach et al. 1999].

Overall, the inflammatory response and cell-mediated immunity could initially be protective in atherogenesis, but persistent and excessive inflammatory responses will favor progression of the disease. Continued inflammation results in enhanced cycles of accumulation of mononuclear cells that proliferate in the plaque and induce the growth of SMC that become intermixed with the area of inflammation to form an intermediate lesion. The formation of fibrous tissue leads to further restructuring of the lesion, so that a fibrous cap, the so-called advanced and complicated lesion, covers it.

I.II. Lipoprotein lipase (LPL)

I.II.1. General

LPL is the major enzyme responsible for the hydrolysis of triglycerides (TG) in chylomicrons and very low-density lipoproteins (VLDL). Studies have shown that inhibition of the enzyme by antisera leads to the accumulation of TG in plasma [Ma et al. 1991, Kyte et al. 1982]. Patients with type 1 hypertriglyceridemia, a rare heritable disease, have decreased plasma LPL activity, with high plasma TG and low HDL concentrations. LPL is mainly synthesized by adipose and skeletal muscle tissues. However, it is also produced by many other tissues including heart, lung, mammary gland, kidney, adrenal gland, testes, ovaries, brain, liver and macrophages. LPL synthesized by these

tissues, is secreted to the cell surface, and transported, by an unknown mechanism, to the capillary endothelial surface where it binds to heparan sulfate proteoglycans (HSPG) [Enerbäck et al. 1987, Cheng et al. 1990]. In the body, LPL plays a directive function, such as fatty acid flux to given tissues, reflecting its activity on the capillary bed of these tissues [Parkin et al. 1982].

I.II.2. LPL properties

I.II.2.1. LPL gene organization

The human LPL gene [Kirchgessner et al. 1989, Deeb et al. 1989] is approximately 30 kb pairs with 10 exons in the mRNA-specifying sequence. Exons 1-9 are 105-276 bp in size, whereas exon 10 with the specific entire 3' noncoding sequence is 1,948 bp in length. The organization of human LPL gene is shown in **Figure 3**.

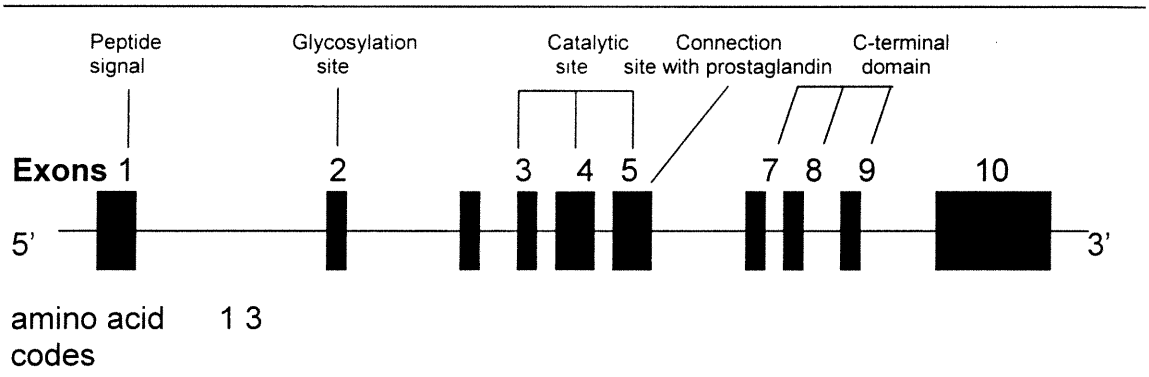


Figure 3. Diagrammatic representation of the LPL gene and its structure-function relationship. [Kirchgessner et al. 1989, Deeb et al. 1989].

I.II.2.2. Structural and functional domains of LPL

I.II.2.2.1 Tertiary structure

At present, the LPL crystal structure is not known. The crystal structure of hepatic lipase [Winkler et al. 1990] can serve as a good model for the crystal structure of LPL. The LPL molecule is folded into two domains. The large N-terminal domain comprises residues 1-336, a typical α/β structure dominated by a central β-sheet. The C-terminal domain is the β-sandwich type with four anti-parallel strands, formed by two layers of the β-sheet. The α-carbon backbone of the molecule is highlighted with the β-structures and disulfide bonds. The N-terminal domain forms a nearly spherical, globular structure, whereas the smaller C-terminal domain is elongated like a bent cylinder, which is attached to the spherical N-terminal domain.

I.II.2.2.2. Apolipoprotein CII (ApoCII)-binding site

Activation of LPL by apoCII is required for maximal LPL activity [Flier et al. 1989]. Under X-ray crystallography of hepatic lipase, the N-terminal peptide appears as part of a cleft structure. Further mapping is required to determine whether the corresponding cleft of LPL is the apoCII-binding site. Because of competitive inhibition of the charged carboxyl terminal tetrapeptide of apoCII [Cheng et al. 1990], the site could also include regions of high positive charge, such as heparin-binding sites [Martin et al. 1988]. This is consistent with the

finding that heparin is a competitive inhibitor of apoCII in LPL activation [Posner et al. 1983].

I.II.2.2.3. Heparin-binding domain

Many studies have shown the importance of the N-terminal domain of the LPL molecule for its binding to HSPG expressed on endothelial cell surfaces [Wong et al, 1991, Santamarina-Fojo et al, 1994]. The heparin-binding domain is located in exon 6 of the human LPL gene [Deeb et al. 1989]. In contrast, the role of the C domain in LPL binding to HSPG is still controversial [Wong et al, 1991, Santamarina-Fojo et al, 1994, Ma et al, 1994].

I.II.2.2.4. Glycosylation sites

Glycosylation is required for the maturation of LPL and the secretion of an active enzyme. There are three N-glycosylation sites in LPL: Asn-43, Asn-257, and Asn-359 [Semb et al. 1989, Semenkovich et al, 1990]. However, only Asn-43 is important for the secretion and the functional activity of the enzyme [Semenkovich et al. 1990], while substitution of Asn-257 and Asn-359 does not appreciably affect LPL activity.

I.II.3. Structure and function of apoCII

I.II.3.1. Active site domain of apoCII

The presence of apoCII increases basal LPL activity. The active-site domain of apoCII for LPL activation has been studied either with proteolytic fragments or synthetic apoCII-peptides. Based on cDNA sequencing, the human apoCII sequence contains 79 amino acids [Jackson et al. 1984, Myklebost et al. 1984]. ApoCII has two methionine residues. One is located at position 9, and the other at position 60. Both the N-terminal ($apoCII_{1-9}$) and the center fragments ($apoCII_{10-60}$) have no effect on LPL activation. Only the C-terminal peptide ($apoCII_{61-79}$) causes a four-fold increase in LPL activity.

I.II.3.2. ApoCII gene mutation

A deficiency of apoCII induces reduction of LPL activity, leading to hyperchylomicronemia, a rare genetic syndrome, described in 1978 by Breckenridge et al. [Breckenridge et al, 1978].

I.II.4. Role of LPL in lipid metabolism

While dietary lipids are transported in the bloodstream as chylomicrons, VLDL are synthesized in the liver. These TG-rich lipoproteins must be enzymatically processed to release free fatty acids (FFA), which are either re-esterified and stored in adipocytes or metabolized as energy sources in peripheral tissues [Robinson 1963, Ladu et al. 1991]. LPL plays a central role in the metabolism of chylomicrons and VLDL.

I.II.5. Mechanisms of LPL regulation

I.II.5.1. Transcriptional regulation

Several studies have demonstrated that *cis* elements present in the promoter of the LPL gene are involved in the transcriptional control of LPL. These elements include the octamer-1 (Oct-1), the glucocorticoid responsive element (GRE), the fat specific element (FSE), the thyroid responsive element (TRE), the activated protein –1 and 2 (AP-1, -2), the stimulatory protein1 (SP1) and the lipoprotein (LP) elements [Enerbäck et al. 1993]. The role of these elements in the regulation of the LPL gene has been examined by in vitro protein/DNA-binding analyses and by transient transfection assays [Hua et al. 1991, Previato et al. 1991, Enerbäck et al. 1992, Currie et al. 1992]. It has been demonstrated that Oct-1 binding to the promoter of the LPL gene is required for the transcription of the gene. Moreover, it has been reported that a transcriptional factor named *NF-Y* binds specifically to the CAAT motif located at position –65 relative to the transcriptional start site [Currie et al. 1992]. T*rans*-acting factors have also been shown to bind to the *cis*-element LP-α (-702 to –666) and LP-β (-468 to -430) [Enerbäck et al. 1992]. These *trans*-acting factors recognize a DNA element in common with the *fork head*/hepatic nuclear factor-3 (HNF-3) family of transcription factors [Lai et al. 1991].

Some experiments have used anti-sense DNA methods to provide evidence of transcriptional regulation of LPL expression. Two transcriptional DNA-binding proteins that play a role in controlling adipogenesis have been examined in this manner. They are c-fos, which is a component of the AP-1

complex [Barcellini-Couget et al. 1993], and the CCAAT-enhancer-binding protein or C/EBP [Samuelsson et al. 1991, Umek et al. 1991, Lin et al. 1992]. Recently, Sartippour et al. [1998] have reported that exposure of macrophages to high glucose sequentially induces PKC activation and its well-known target, *c-fos*, which, by interacting with the AP-1 sequence, leading to increased transcription of the LPL gene.

In transfection studies, deletion constructs have served to determine that both positive and negative regulatory elements exist within the 5' flanking region of the LPL promoter [Previato et al. 1991, Enerbäck et al. 1992]. In stable transfection experiments, it has been found that linkage of either the LP-α or LP-β sequence to a constitutively expressed promoter is sufficient to increase the reporter gene expression [Enerbäck et al. 1992].

Recently it has been demonstrated that activators of peroxisome proliferator-activated receptors (PPAR) alpha (α) and gamma (γ) regulate the transcription of the LPL gene in adipocytes [Schoonjans et al. 1996a]. This effect results from the formation of PPAR/retinoid X receptor (RXR) heterodimers and the binding of this complex to the cis-acting regulatory PPAR response element (PPRE) localized in the LPL promoter [Schoonjans et al. 1996b].

I.II.5.2. Post-transcriptional regulation

I.II.5.2.1 Stability of LPL mRNA

Little is known about the stability of LPL mRNA. White et al have reported that lipopolysaccharide (LPS) decreases the stability of human monocyte/macrophage LPL mRNA [White et al, 1988]. IFNγ also decreases by 50 % the stability of LPL mRNA in human monocytes/macrophages [Jonasson et al, 1990]. Recently, Ranganathan et al have demonstrated that translation of adipocyte LPL is regulated by a catecholamine-activated protein that binds to the 3` untranslated region of the LPL mRNA [Ranganathan et al, 1997]. PKC-induced phosphorylation of this protein has been suggested to be involved in the control of LPL translation [Ranganathan et al, 1998].

I.II.5.2.2. Glycosylation

There are three putative sites for N-linked glycosylation (Asn-X-Ser/Thr) located at Asn-43, Asn-257, and Asn-359 in the cDNA sequences of human, murine, and guinea pig LPL [Semb et al. 1989]. Glycosylation of LPL at the first N-linked glycosylation site is essential for catalytic activity and secretion [Semenkovich et al, 1990]. A number of studies have demonstrated the importance of glycosylation using inhibitors of glycosylation and inhibitors of glycoprotein transfer from the rough endoplasmic reticulum (RER) to the Golgi [Ben-Zeev et al. 1992, Simsolo et al. 1992, Casrroll et al. 1992, Masuno et al. 1992].

I.II.5.2.3 Heparin-binding activity

An additional post-translational regulatory site is provided by the interaction of secreted LPL with cell surface HSPG. This pathway, with LPL bound on the cell surface, then either released into serum or internalized and degraded within the lysosomal compartment of the cell, provides a rapid mechanism for modulating plasma LPL levels [Cisar et al. 1989]. Binding of LPL to endothelial cells is heparin-sensitive indicating thereby that HSPG mediate this interaction [Shimada et al. 1981, Friedman et al. 1982]. There are two proteins, which may be responsible for the binding and transport of LPL within endothelial cells. One is a HSPG of 220 KD with a protein core of 50 kDa [Saxena et al. 1991], the other is a 116 kDa heparan releasable protein with a high affinity for both LPL and proteoglycans [Sivaram et al. 1992]. Evidence has recently been provided that transcytosis of LPL across endothelial cells requires both HSPG and the VLDL receptor [Obunike et al. 2001].

I.II.6. Physiological regulators

I.II.6.1. Nutrients

It is well-known that nutritional status is the first physiological regulator of LPL activity in the adipose and muscle tissues. In contrast, there are few data at present on the effects of fasting and refeeding on macrophage LPL control.

It has been demonstrated that in normal or lean subjects within 20% of ideal body weight, adipose LPL levels decrease up to 75% with caloric restriction [Taskinen et al. 1979, Lithell et al. 1978, Farese et al. 1991, Iverius et al. 1985].

In contrast, muscle LPL activity increases in the fasting state [Farese et al. 1991, Olivecrona et al, 1995]. In the post-prandial state, adipose LPL activity increases whereas muscle LPL activity decreases. Regulation of LPL in the fasting and post-prandial states has been postulated to involve post-translational mechanisms.

Glucose is a nutritional factor that modulates the expression of macrophage LPL expression. Indeed, Sartippour et al. [1998] reported that macrophages cultured in the presence of high glucose concentrations overproduce LPL. The regulation of macrophage LPL gene expression by glucose is exerted at the transcriptional level and implies the activation of PKC and the protooncogene c-fos [Sartippour et al. 1998].

LPL generates fatty acids by its hydrolytic action and evidence has been provided that LPL binds fatty acids [Edwards et al, 1994]. High level of fatty acids in the plasma facilitates the dissociation of LPL from its connection sites [Karpe et al. 1992, Peterson et al. 1990] and thereby regulates the release of LPL from the endothelium. Fatty acids are significant energy substrates for macrophages [Yin et al. 1997, Newsholme et al. 1987] and may therefore regulate macrophage LPL expression. This hypothesis has been supported by the findings that omega-3 fatty acids decrease the expression and production of LPL by murine macrophages [Renier et al. 1993] and that saturated and unsaturated fatty acids directly regulate murine macrophage LPL expression, both at the gene and protein levels [Michaud et al. 2001]. This effect was found to involve PPARs.

I.II.6.2. Hormones

I.II.6.2.1. Steroids

The in vitro effect of steroid compounds has been examined on both adipocyte and macrophage LPL expression. It has been shown that, in primary cultures of murine macrophages and in the macrophage-like J774 cell line, dexamethasone inhibits LPL activity [Goldman 1990, Goldman et al. 1989] and LPL secretion [Behr and Kraemer, 1986]. In vivo studies suggest that LPL levels are higher in female than in male rats. Sex steroids could account for this difference [Eisenbäck and Gimble, 1993]. Progesterone administration to female rats increases adipocyte LPL activity, while estrogens decrease this parameter [Gray and Wade, 1980, Pedersen et al, 1992].

I.II.6.2.2. Insulin

Insulin infusion is associated with increased adipose tissue LPL levels in normal subjects [Sadur et al. 1982]. The effect of insulin on adipocyte LPL seems to be exerted at the post-transcriptional level. Indeed, several studies have shown that insulin increases LPL protein synthesis by 42-48%, but has no influence on either total LPL mRNA levels or the LPL transcription rate [Samuelsson et al. 1991, Semenkovich et al. 1989]. The effect of insulin on macrophage LPL regulation has also been evaluated. Behr et al. [1988] found that insulin does not exert a direct effect on LPL secretion by macrophages, although these authors demonstrated that insulin deficiency causes a profound decrease in macrophage LPL secretion. In our laboratory we have obtained

evidence that hyperinsulinemic subjects overproduce macrophage LPL [Sartippour and Renier, 2000]. These observations are in contrast with those of Dobrian et al. [2000] demonstrating that macrophages from non-insulin-dependent-diabetes-mellitus patients display a decrease of LPL mRNA expression with a reduction of secreted LPL activity.

I.II.6.2.3. Growth hormone (GH)

Post-heparin plasma LPL activity decreases in hypophysectomized rats, and this effect is reversed by GH therapy. GH transiently activates c-fos gene expression by a PKC-dependent pathway and modulates the expression of the LPL gene in Ob1771 adipose cells [Pradines-Figuères et al. 1990].

Ottosson et al. [1995] found that GH counteracts the potent stimulatory effect of glucocorticoids on LPL activity without affecting LPL mRNA levels. Therefore, they suggested that the inhibition of LPL activity by GH probably occurs during translation and/or post-translational processing of the enzyme.

I.II.6.3. Pro-inflammatory cytokines

The regulation of LPL by cytokines is of potential crucial importance in determining the levels of LPL in the arterial wall and thereby the development and/or progression of the atherosclerotic lesions.

I.II.6.3.1. TNF-α

Accumulating evidence suggests that TNF-α affects circulating lipids as well as vascular wall biology and exerts potent pro-atherogenic effects. Indeed, TNF-α has been shown to stimulate new vessel formation [Leibovich et al. 1987] and to induce hemorrhagic necrosis [Watanabe et al. 1988], features characteristic of evolving atheroma. In addition to favoring leukocyte adhesion to endothelial cells [Gamble et al. 1985], this cytokine also promotes tissue factor-like procoagulant activity and suppresses endothelial cell surface anticoagulant activity [Bevilacqua et al. 1986]. It is expressed by macrophages in human atheroma [Barath et al. 1990, Rus et al. 1991] and plays a critical role in the induction of insulin resistance, a condition associated with increased vascular risk. This cytokine has been found to diminish LPL gene expression and activity in J774 macrophages. A maximal inhibitory effect of this cytokine on macrophage LPL expression was observed in presence of IFNγ [Tengku-Muhammad et al. 1996, 1998]. In contrast, no effect of TNF-α on human macrophages was observed [White et al. 1998]. Because this cytokine suppresses adipocyte LPL expression, it has been postulated that decreased LPL expression in animals

with visceral obesity may result from increased levels of TNF-α. [Hikita et al. 2000].

I.II.6.3.2. IL-1

IL-1 is a prominent pro-inflammatory cytokine in a multitude of systemic inflammatory states. Studies aimed at determining the role of IL-1 on LPL expression demonstrate that IL-1 decreases murine macrophage LPL expression [Tengku-Muhammad et al. 1996, 1999]. In contrast, no effect of this cytokine was reported on human macrophage LPL activity [Querfeld et al. 1990].

I.II. 6.3.3. IFN-γ

Accumulating evidence demonstrates that IFN-γ inhibits adipocyte and macrophage LPL activity [Patton et al. 1986, Ogawa et al. 1989, Beutler et al. 1985, Price et al. 1986, Grégoire et al. 1992]. In human monocytes/macrophages, IFN-γ causes a decrease of LPL activity [Querfeld et al. 1990, Jonasson et al. 1990]. Tengku-Muhammad et al. [1996, 1998, 1999] demonstrated that this effect is exerted at the level of LPL mRNA metabolism (decreased transcription or RNA stability) and that co-exposure of macrophages to IFN-γ and LPS or TNF-α results in a synergistic suppression of LPL activity.

I.II.6.3.4. IL-6

IL-6 is a potential mediator of the lipogenic effects of TNF-α [Grunfeld et al. 1990]. It is released systemically in response to TNF-α, IL-1, or LPS. IL- 6 has been documented to reduce tissue LPL activity and may play a role in inducing cancer cachexia [Greenberg et al. 1994]. It also has been found that administration of IL-6 to mice in vivo resulted in a 50% decrease in adipose tissue LPL activity [Grunfeld et al. 1990]. In contrast no effect of IL-6 on macrophage LPL activity or LPL mRNA levels was found [Tengku-Muhammad et al.1996].

I.II.6.3.5. Leukemia inhibitory factor (LIF)

LIF, a cytokine released by tumor cells, was initially identified on the basis of its ability to induce differentiation in a monocytic leukemia cell line [Hilton et al. 1991]. In vitro research has established its ability to decrease LPL activity in murine 3T3-l1 adipocytes [Mori et al. 1989, Kawakami et al. 1991]. This cytokine has no effect on macrophage LPL activity or LPL mRNA levels [Tengku-Muhammad et al.1996].

I.II.6.3.6. IL-11

The original IL-11 cloned from bone marrow stromal cells had the ability to stimulate the proliferation of an IL-6-dependent plasmacytoma (B-cell) clone [Paul et al. 1990]. It has since been demonstrated that IL-11 inhibits LPL activity

in adipocytes [Kawashima et al. 1991, Ohsumi et al. 1994]. The effect of IL-11 on macrophage LPL has been studied in the murine J774.2 cell line [Tengku-Muhammad et al. 1996, 1998, 1999]. These authors found that IL-11 exerts an inhibitory effect on macrophage LPL expression.

I.II.6.3.7. LPS

LPS inhibits in vivo adipocyte LPL activity. This effect may be exerted at the post-transcriptional level [Gouni et al. 1993]. The suppressive effect of LPS on adipose LPL activity could involve the secretion of cytokines by macrophages including IL-1, IL-6, IFN-γ and TNF-α [Cornelius et al. 1988, Greenberg et al. 1992]. This possibility is supported by the findings that LPS inhibits both murine and human macrophage LPL activity in vitro [White et al. 1988, Sopher et al. 1987]. Recently, Tengku-Muhammad et al. [1999] used J774.2 macrophages to demonstrate that both the tyrosine kinase and phosphatidylinositol-3'-kinase signaling pathways are involved in the inhibitory effect of LPS and cytokines on macrophage LPL expression.

I.II.6.4. Prostaglandins (PGs)

PGs are produced in multiple cell types, including adipocytes, through arachidonic acid metabolism via the cyclo-oxygenase pathway. $PGF_2\alpha$ alone in either primary rat adipocytes or murine pre-adipocytes inhibits LPL mRNA expression [Serrero et al. 1992]. In macrophages, an inhibitory effect of PGE_2 has been reported on macrophage LPL mRNA levels, mass and activity [De

Sanctis et al. 1994]. This effect is mediated by an increased production of cAMP. PGs have also been found to mediate LPS-induced inhibition of macrophage LPL expression [De Sanctis et al. 1994]. Recently, Obunike et al. [2000] have reported that enhanced LPL gene expression results in increased PG production.

I.II.7. LPL in diabetes

Diabetes is an independent risk factor for cardiovascular disease. LPL, a potential atherogenic protein, has been found to undergo significant functional alterations in the diabetic state.

Non-insulin-dependent diabetic patients have higher plasma TG and lower HDL cholesterol concentrations compared to normal subjects. This diabetic dyslipidemia is associated with low plasma LPL activity [Murata et al. 1988, Ishibashi et al. 1989, Rapp et al. 1994]. Whereas diabetes has been repeatedly shown to decrease adipose LPL activity, LPL activity in skeletal muscle of diabetic patients has been found to be either decreased or unchanged [Farese et al. 1991]. It is well-documented that LPL regulation is tissue- and cell-specific [O'Brien et al. 1994]. Upregulation of macrophage LPL secretion has been found in diabetes-prone mice [Corey et al. 1997] and in type II diabetic patients [Sartippour et al. 2000]. It has been proposed that the increase in macrophage LPL in human diabetes could be due to peripheral factors such as high glucose and fatty acids [Sartippour et al. 1998, Michaud and Renier, 2001].

I.II.8. LPL in atherosclerosis

LPL, which is synthesized by adipose cells, SMC, and macrophages [O'Brien et al. 1992] plays a central role in lipid metabolism. Depending on its location, LPL has been suggested to play a dual role in atherogenesis [Williams et al. 1992, Mead et al. 1999, Santamarina-Fojo et al. 2000].

I.II.8.1. Pro-atherogenic effect of LPL

The contribution of LPL to atherogenesis is significantly influenced by the balance between plasma LPL activity and vessel wall protein. Indeed, it has been demonstrated that LPL activity in plasma decreases vascular risk while LPL produced by macrophages in the vascular wall promotes atherosclerosis. Evidence for a pro-atherogenic role of macrophage LPL has been provided on the basis primarily of in vitro and ex vivo studies. It has been shown that LPL is synthesized by macrophages in the atherosclerotic lesion [Yal-Herttuala et al. 1991, O'Brien et al. 1992] and that macrophage LPL expression is positively associated with atherosclerotic susceptibility in inbred mice [Renier et al. 1993]. LPL content in the arterial wall has also been reported to increase with the progression of the lesion [Corey et al. 1977] and a correlation has been documented between its accumulation in the arterial wall and that of cholesterol ester [Zilversmit 1979]. LPL also functions as a ligand, associating with lipoproteins and promoting their binding to specific receptors expressed on cell surface. It may promote atherogenesis by increasing the binding and retention of LDL by proteoglycans of the subendothelial matrix [Lindqvist et al. 1983,

Ishibashi et al.1990, Eisenberg et al.1992, Rumsey et al. 1992, Beisiegel et al.1991, Mulder M et al.1992, Williams et al. 1992, Nykjaer et al.1993, Chappell et al. 1993, Saxena et al.1992, Williams and Tabas 1995]. The atherogenic ligand function of LPL is increased by LDL oxidation [Auerbach et al. 1996, Kaplan et al. 1997, Makoveichuk et al. 1998]. LPL also exerts its pro-atherogenic effects by acting as a monocyte adhesion protein [Obunike et al 1997, Mamputu et al. 1997]. Finally, LPL-mediated hydrolysis of VLDL produces LDL, a major contributor to the development of atherosclerotic lesions [Brown et al. 1983, Steinberg et al. 1989]. Recently, the pro-atherogenic effect of macrophage LPL has been confirmed in vivo [Babaev et al 1999, 2000, Van Eck et al. 2000].

I.II.8.2. Anti-atherogenic effect of LPL

Accumulating evidence supports the view that plasma LPL plays an anti-atherogenic role. Firstly, Benlian et al. [1996] have recently reported that several LPL-deficient patients develop relatively advanced atherosclerosis. Additionally, it has been found that individuals who are heterozygous for LPL mutations that reduce LPL enzymatic activity are predisposed to premature atherosclerosis [Henderson et al. 1999]. Conversely, a variant of LPL that is associated with increased plasma activity has been found to protect against atherosclerosis [Gagne et al, 1999]. Recently, pharmaceutical interventions intended to elevate HDL resulted in increased plasma LPL expression and protected against atherosclerosis [Tsutsumi et al. 1995, 1997]. Furthermore, in the liver, it has been demonstrated that LPL overexpression can normalize the atherosclerotic

lipoprotein profiles of both apoE-deficient and LDL receptor-deficient mice, and protect wild-type mice against diet-induced hyperlipidemia [Shimada et al. 1993, 1996, Zsigmund et al. 1997, Yagyu et, 1999].

I.III. PPARs

I.III.1. General

PPARs belong to the steroid/thyroid/retinoid receptor superfamily. They represent nuclear lipid-activated receptors that control a variety of genes involved in several pathways of lipid metabolism, such as fatty acid transport, intracellular binding and activation, as well as catabolism (β-oxidation and omega–oxidation). PPARs also influence cellular proliferation, differentiation and apoptosis and play a role in the control of inflammation.

I.III.1.1. PPAR structure and function

PPARs are compact molecules. Human PPARα has 468 amino acid residues compared with 441 for PPARβ/δ and 479 for PPARγ. PPARs have five or six structural regions (A-F) in four functional domains. The C domain is DNA-binding (DBD), and the E/F domain is ligand-binding, which plays a key role in transduction of the hormonal signal into transcriptional activation. Intramolecular communication takes place between the A/B NH_2-terminal and the DBD carboxy-terminal, where hormones bind to PPARγ. Activation of PPARγ can be depressed by phosphorylation of a seryl residue in A/B, mediated by MAPK [Shao et al.

1998], whereas phosphorylation of PPARα positively affects receptor/ligand affinity [Shalev et al. 1996]. Phylogenetic studies indicate that the E domain in PPARs has evolved three times faster than in thyroid and trans-retinoic acid (RAR) receptors [Dreyer et al. 1993]. Perhaps this may explain PPARα and γ differences and opposing metabolic pathways.

I.III.1.2. Molecular mechanism of action of PPARs

PPARs constitute a nuclear receptor subfamily. Three isoforms of PPARs, termed α, β (also called δ or NUC1) and γ, are each encoded by a separate gene and expressed by distinct tissues [Braissant et al. 1995, Auboeuf et al. 1997, Granneman et al. 1998]. Their developmental distribution patterns [Braissant et al. 1998] also have been described. PPARs are ligand-activated transcription factors, binding upon heterodimerization with RXRs to PPRE in the promoter of target genes [Schoonjans et al. 1996]. PPAR consensus PPREs consist of a direct repeat of nuclear receptor hexameric AGGTCA recognition sites (DR-1 and DR-2) [Gervois et al. 1999]. The DR-1 pattern is specific to the PPAR/RXR heterodimer **(Fig. 4)**. DR-1 is clearly different from DR-3 (estrogen receptor, vitamin D receptor), DR-4 (thyroid hormone receptor) and DR-5 (RAR S-γ RAR), which are response elements of other nuclear receptors.

Recently, it has been shown that PPARs can also repress gene transcription by a combination of protein-protein interactions and cofactor squelching without binding to PPRE. PPARs can negatively interfere with the NF-κB, STAT and AP-1 signaling pathways in a DNA-binding independent manner

[Chinetti et al. 1998, Ricote et al. 1998, Stael et al. 1998, Zhou et al. 1999, Delerive et al. 1999]. Furthermore, PPARs interact with AP-1 and NF-κB proteins, including c-Jun [Sakai et al.1995] and P65 [Delerive et al. 1999], interfering with their target sequences [Delerive et al. 1999, Marx et al. 1999]. PPARs also interfere negatively with retinoic acid and GC receptors, which are also nuclear receptor family members [Kamei et al. 1996].

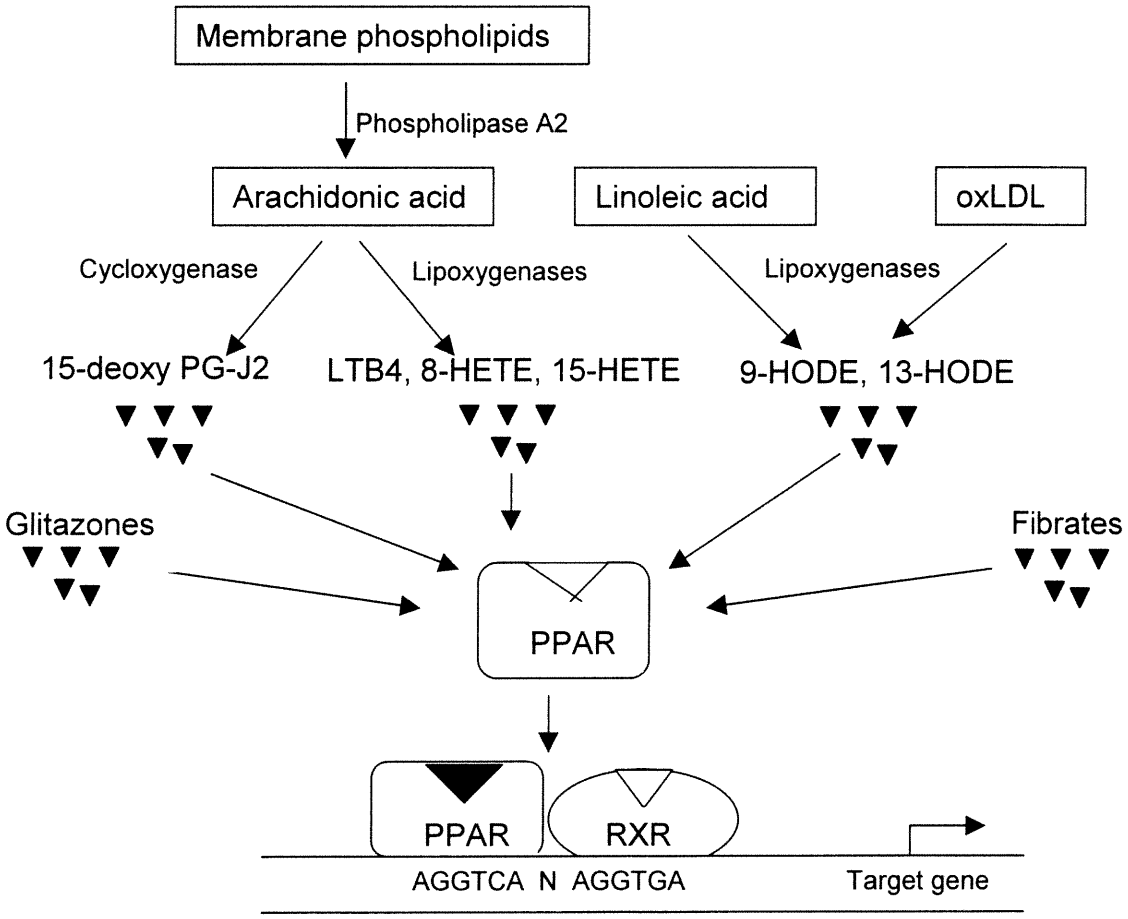


Fig. 4. Peroxisome proliferator-activated receptors : ligands and transcriptional activation [Chinetti et al. 2000].

I.III.1.3. Tissue distribution of PPARs

Studies of PPARs tissue distribution have been restricted to the analysis of its mRNA distribution. PPARα is predominantly expressed in liver, heart, kidney and brown adipose tissue. PPARβ is abundantly expressed in various tissues, in almost the same distribution as PPARα. PPARγ1 and PPARγ2 are expressed in a tissue-specific manner. PPARγ1 exhibits a distribution pattern reminiscent of PPARα. PPARγ2 is predominantly expressed in adipose tissue. The differential tissue distribution of PPARs has been well-documented **(Table 1).**

Table 1. Relative tissue expression of PPAR isoforms

Tissue	PPARα	PPARβ	PPARγ1	PPARγ2
Heart	+ + +	+ + +	+ + +	+ +
Liver	+ + +	+/-	+ + +	+/-
White adipose tissue	-	+ +	-	+ + + +
Brown adipose tissue	+ + +	+ +	-	-
Lung	-	+ + +	+	+ +
Spleen	-	+ + +	+/-	+ +
Kidney	+ + +	+ +	+ + +	+
Brain	-	+ + +	+	+
Intestine	+	+ + +	-	-
Adrenal	-	+ + +	-	-
Muscle	+	+ + +	+	+
Thymus	+	-	-	-
Testis	+	+/-	+	-
Pancreas	-	-	-	-

I.III.1.4. Main characteristics of PPARα and PPARγ (Table 2)

Table 2.

PPARα	PPARγ
Mainly liver (also present in other tissues)	Mainly adipose tissue and colon
Stimulation of β-oxidation · LPL metabolism	Stimulation of fat storage
Inflammation (stimulates breakdown of inflammatory fatty acids)	Glucose and lipid metabolism
	Fat formation
Leukotriene B4, 8-S-HETE, and fibrates	Prostaglandin J2 and thiazolidinediones

I.III.1.5. Natural and synthetic ligands of PPARs

PPARs can be activated by a number of ligands, which include natural and synthetic groups. There is firm evidence that fatty acids are PPAR natural ligands interacting directly with the nuclear receptor for at least part of their PPAR-dependent transcriptional activity. Polyunsaturated fatty acids bind to all three PPAR isoforms with varying affinities, though saturated fatty acids preferentially bind PPARα **(Table 3)**.

Synthetic ligands include some hypolipidemic and anti-diabetic agents. For example, clofibrate and WY-14643 belong to the hypolipidemic fibrate group binding preferentially to PPARα. Thiazolidinediones (TZDs), which include troglitazone, pioglitazone, and BRL49653 (rosiglitazone), are a class of antidiabetic drugs that are structurally derived from clofibric acid but selectively bind PPARγ [Lehmann et al. 1995, Berger et al. 1996] **(Table 4)**.

Table 3. PPAR natural ligands

Natural ligands	PPARα				PPARβ				PPARγ			
	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA
Unsaturated fatty acids												
ω3-PUFAs												
α-Linolenic C18:3	+	+	+		+/-	+/-	+		+		+	
γ -Linolenic C18:3	+	+			+				+			
Eicosapentaenoic C20:5(EPA)	+	+	+		+	+/-	+		+		+	
Docohexaenoic C22:6 (DHA)		+	+			+/-	-				+	
ω6-PUFAs												
Linoleic C18:2	+	+	+		+/-	+			+		+	
Dihomo-γ–λlinolenic C20:3	+				+				+			
Arachidonic C20:4	+	+	+		+	+			+		+	
ω9-mUFAs												
Palmitoleic C16:1	+				+				+			
Oleic C18:1	+		+		+		-/+		+		-	
Elaidic C18:1trans							-/+				-	
Erucic C22:1	-	-	-/+		-		-/+		-		-	
Nervonic C24:1		-	-				-/+				-	
ω2-mUFA												
Petroselinic C18:1	+		+				-/+		+			
Saturated fatty acids												
Capric C10:0	-				-				-			
Lauric C12:0	-	+/-			-				-			
Myristic C14:0	+	+/-			-/+				-/+			
Palmitic C16:0	+	+			+				-			
Stearic C18:0	+				+				-			
Arachidic C20:0	-				-				-			
Behenic C22:0	-				-				-			
Dicarboxylic fatty acids												
Dodecanedoic C12			-				-				-	
Eicosanoids												
±8-HEPE(hydroxyeicosapentaenoic)		+										
±8-HETE		+					-					
8S-HETE	+	+	+				+/-			-/+	-/+	
8R-HETE	-	-	+/-				-			-	-	
8(9)-EpEtrE		-										
±8-HETrE (hydroxyeicosatrienoic)		-/+										
12-HETE		-										
Leukotriene B4		-	+									
9-HODE(9-hydroxyoctadenoic acid)									+			
13-HODE									+			
15-Deocy-12, 14-PGJ2		-	-			-	-		+	+	+	

Direct interaction between natural and synthetic compounds and PPARs was analyzed by the following approaches : saturation binding assay (SABA), Competition binding assay (COBA), PPAR :RXR-DNA ligand –induced complex formation (LIC), coactivator-dependent receptor binding assay (CARLA), and differential protease sensitivity assay (DPSA). +, binding detected easily; +/-, weak binding; -/+, very weak binding detected; -, compound tested but no binding detected. [Desvergne et al. 1999]

Table 4. PPAR synthetic ligands

Synthetic ligands	PPARα				PPARβ				PPARγ			
	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA	SABA or COBA	LIC	CARLA	DPSA
Prostaglandin 12 analogs												
Carbaprostacyclin (cPGI)		+				+						
Iloprost		+				+						
Cicaprost		-				-						
Leukotriene B4 analogs												
Trifluoromethyl leukotriene B4			+									
ZK 151657			+				+/-				-	
ZK 158252			+				-				-	
Leukotriene D4 antagonist												
Ly 171883		+	+									
Hypolipidemic agents												
Clofibric acid		+	+	+		-						
Ciprofibric acid		+	-			-	-				+	
Bezafibric acid(xPPARs)			-				+				-	
Fenofibric acid			-				-				-	
Pirinixic acid (Wy-14643)		+	+			-	-				-	
GW 2331 (fibrate analog)	+								+			
GW 2433 (fibrate analog)	+				+							
Eicosatetraynoic acid (ETYA; Arachidonic analog)			+	+			+					
Hypoglycemic agents (thiazolidinediones)												
Rosiglitazone (BRL 49653)	-	-	-			-	-		+		+	+
AD-5075									+			+
Troglitazone									+			
Hypolipidemic and hypoglycemic agents (nonthiazolidinedione)												
L-165041					+				-/+			
L-165461					+				+/-			
L-783483					+				+/-			
L-796449					+				+			
Nonsteroidal antiinflammatory drugs (NSAIDs)												
Indomethacin									+			
Flufenamic acid									+			
Fenoprofen									+			
Ibuprofen									+			
Carnitine palmitoyl transferase I (CPT1) inhibitors												
Ly-171883		+		+								
2-Bromopalmitate (2Br-C16)		+										
Tetradecylglycidic acid (TDGA)		+										
Fatty acyl-CoA dehydrogenase inhibitors												
Ortylthiopropionic acid (OTP)		+										
Tetradecylthippropinic acid (TTP)		+										
Nonylthioacetic acid (NTA)		+										
Tetradecylthioacetic acid (TTA)		+										

I.III.1.6. Regulation of PPAR expression by cytokines

It is known that PPAR expression is under the control of a wide variety of inflammatory cytokines. A recent study has shown that treatment with different glitazones can reverse the inhibitory effect of PPARγ expression induced by TNFα, IL-1α, IL-1β, and IL-6 in mature rat adipocytes [Tanaka et al. 1999]. Conversely, IL-4, which exerts an anti-inflammatory action through macrophage inactivation, induces PPARγ1 expression in monocytes and macrophages [Huang et al. 1999]. In addition, 9- and 13-HODE, which are pro-inflammatory mediators derived from oxLDL increase PPARγ mRNA levels in human macrophages [Tontonoz et al. 1998].

I.III.2. Role of PPARs in lipid-glucose metabolism and inflammation

Since the first characterization of PPARs [Issemann et al. 1990], their importance in metabolic regulation has become clear. It has been established that treatment with clinical hypolipidemic drugs (fibrates) efficiently decreases plasma triglyceride levels and increases HDL cholesterol concentration in diabetic patients [Staels et al. 1998b]. In the liver and in cultured hepatocytes, fibrates activate PPARα, enhancing fatty acid β-oxidation, decreasing apoC-III production, and lowering TG and VLDL secretion [Schoonjans et al. 1996a, 1996b, 1997]. Recently, the role of PPARα in HDL and triglyceride metabolism as well as in the hepatic regulation of apolipoprotein and fatty acid β-oxidation enzyme expression has been further demonstrated unequivocally [Peters et al.

1997, Aoyama et al. 1998]. Peters et al. [1997] has demonstrated that administration of the fibrate Wy 14,643 to wild-type mice results in marked depression of hepatic apolipoprotein C-III mRNA and serum triglycerides compared with untreated controls. Aoyama et al. [1998] has showed that PPARα modulates constitutive expression of genes encoding several mitochondrial fatty acid-catabolizing enzymes in addition to mediating inducible mitochondrial and peroxisomal fatty acid beta-oxidation, thus establishing a role for the receptor in fatty acid homeostasis.

In contrast, PPARγ ligands (glitazones), which are antidiabetic agents, enhance triglyceride catabolism, resulting in decreased plasma TG and FFA levels by the induction of LPL gene expression in adipose tissue [Lefebvre et al. 1997]. Glitazones also regulate important proteins involved in glucose utilization and insulin resistance/tolerance of adipose tissue [Kubota et al. 1999].

In addition to their effects on extracellular lipid metabolism, PPARs act as regulators of cellular fatty acid uptake and control their intracellular fate. Fatty acid transport protein (FATP) [Martin et al. 1997] and fatty acid translocase (FAT/CD36) [Tontonoz et al. 1998, Abumrad et al. 1993] regulate the import and export systems of intracellular fatty acid concentrations. Expression of long chain fatty acid transporters is induced in a tissue-dependent fashion in the liver by PPARα and in adipose tissue by PPARγ activators [Martin et al. 1997, Motojima et al. 1998]. Fibrates also enhance the effect of acyl-CoA synthetase (ACS), a key enzyme in fatty acid esterification which prevents fatty acid efflux from cells of this enzyme in the liver and kidney [Martin et al. 1997, Schoonjans et al. 1993].

Furthermore, PPARα activators enhance carnitine palmitoyltransferase type I (CPT-I), a key enzyme in mitochondrial fatty acid catabolism, which contains a PPRE in its promoter region [Brandt et al. 1998, Mascaro et al. 1998].

PPARα plays also an important role in the enhanced degradation of lipid-derived inflammatory mediators through β-oxidation pathways. This is consistent with the observation that inflammatory response to leukotrienes B4 (LTB4) is prolonged in mice with targeted disruption of the PPARα gene [Devchand et al. 1996].

I.III.3. Role of PPARs in atherosclerosis

Formation of atherosclerotic lesions entails the recruitment and activation of different vascular cell types, such as monocyte/macrophages, endothelial cells, SMC, and T lymphocytes, in the intima of arteries. It has been shown that atherosclerotic lesions and macrophage foam cells express PPARα and γ [Marx et al. 1998, Tontonoz et al. 1998, Ricote et al. 1998b, Staels et al. 1998a, Chinetti et al. 2000], suggesting that PPARs may affect the atherosclerotic process.

PPARα plays an important role in the metabolism of fatty acid, lipids, and lipoproteins. Decreased plasma TG levels in response to fibric acids involve increased expression of genes involved in fatty acid-β oxidation, including LPL and apoC-III [Fruchart et al. 2001].

Experimental evidence implicates this PPAR isoform in inflammation and atherogenesis. Indeed, evidence has been provided that PPARα-deficient mice exhibit a prolonged inflammatory response as compared to control mice

[Devchand et al. 1996] and that PPARα activation inhibits the transcription of several inflammatory response genes. Although an anti-atherogenic effect of PPARα is suggested by the finding that treatment with PPARα activators reduces the progression of atherosclerotic lesions [Frick et al. 1997, Ericsson et al. 1996, Saitoh et al. 1995], evidence that PPARα activation induces macrophage apoptosis [Chinetti et al. 1998] and enhances LPS-induced plasma TNF-α concentrations [Hill et al. 1999] also suggest possible pro-atherogenic effects of this PPAR isoform.

In recent years, many studies have focused on the role of PPARγ in atherosclerosis. It has been found that PPARγ is a crucial component of the innate immune system. Indeed, although PPARγ is barely present in undifferentiated resting monocytes [Tontonoz et al. 1998, Greene et al. 1995, Ricote et al. 1998b], its expression increases strongly during macrophage differentiation [Tontonoz et al. 1998, Ricote et al. 1998b]. The strong expression of PPARγ in macrophage foam cells and atherosclerotic lesions [Ricote et al. 1998b, Marx et al. 1998] suggests that PPARγ may exert pro-atherogenic activity. Several pro-atherogenic effects of PPARγ have been described. For example, it has been shown that activation of the PPARγ/RXR heterodimer enhances macrophage differentiation [Tontonoz et al. 1998] and the uptake of oxLDL by macrophages [Tontonoz et al. 1998, Nagy et al. 1998]. Increased expression of ICAM-1 by PPARγ has also been documented [Chen et al. 1999] and induction of this isoform by LPS has been reported [Leininger et al. 1999]. In contrast, PPARγ may act as an anti-atherogenic factor. This possibility is supported by the

observations that PPARγ inhibits the expression of activation- and differentiation-dependent genes in macrophages [Tontonoz et al. 1999, Ricote et al. 1998b, Jiang et al. 1998], interferes with vascular SMC proliferation [Li et al. 2000] and inhibits the expression of MMP-9 and of various pro-inflammatory cytokines in human macrophages [Marx et al. 1998, Su et al. 1999, Thieringer et al, 2000]. Moreover, it has been shown that the PPARγ-specific agonists rosiglitazone and GW7845 strongly inhibited the development of atherosclerosis in LDL receptor–deficient male mice, despite increased expression of the CD36 scavenger receptor in the arterial wall [Li et al. 2000] and that the PPARγ agonist troflitazone decreases intimal thickness in human carotid arteries [Law et al. 1998].

I.III.3.1. Effect of PPARs on vascular endothelial cells

Vascular endothelial cells contribute to the release of anti-atherogenic mediators in large blood vessels. Endothelial damage or dysfunction is considered to be one major mechanism for the development of atherosclerosis. It has been well-documented that vascular endothelial cells express PPARα, PPARδ, and PPARγ [Inoue et al. 1998, Bishop-Bailey et al. 1999] but the role of PPARδ in endothelial cells has not been well determined yet.

PPARα inhibits the AP-1 signaling pathway, which is involved in thrombin-induced activation of endothelin-1 production by human endothelial cells [Delerive et al. 1999]. In addition, PPARα activators suppress TNF-α-induced VCAM-1 expression partly through inhibition of the NF-κB pathway in human

endothelial cells [Marx et al. 1999]. Recently, PPARα has been shown to repress monocytic recruitment to early atherosclerotic lesions by inhibiting TNF-α-induced VCAM-1 expression [Marx et al. 1999c]. Moreover, it has been demonstrated that activation of PPARα by the synthetic ligand WY14,643 stimulates the synthesis of IL-8 and MCP-1 by human aortic endothelial cells [Lee et al. 2000].

The role of PPARγ in modulating endothelial function has been well-documented. It has been shown that PPARγ activators inhibit the endothelial cell release of endothelin-1 [Satoh et al. 1999, Delerive et al. 1999], a potent vasoconstrictor and vascular smooth muscle cell mitogen [Ruschitzka et al. 1997]. In contrast, these agonists have also been found to increase plasminogen activator inhibitor-1 (PAI)-1 expression [Loskutoff et al. 1997], a well known risk factor of myocardial ischemia and thrombosis. Finally, PPARγ activators can inhibit angiogenesis by inducing apoptosis through a caspase-3-mediated process [Bishop-Bailey et al. 1999] and reducing the vascular endothelial growth factor (VEGF) receptors Flt-1 and Flk/KDR on the endothelial cell surface [Xin et al. 1999].

I.III.3.2. Effect of PPARs in vascular SMC

Vascular SMC also express PPARα, PPARγ, and PPARδ [Staels et al. 1998, Marx et al. 1998, Ricote et al. 1998]. PPARα ligands repress the NF-κB signaling pathway, resulting in the inhibition of inflammatory responses in vascular SMC [Staels et al. 1998]. This mechanism, may explain the inhibitory

effect of fenofibrate and WY-16,463 on IL-1-induced IL-6 production and inducible cyclo-oxygenase (COX-2) expression in vascular SMC [Staels et al. 1998]. Clinical observations demonstrate that hyperlipidemic patients treated with fenofibrate have reduced circulating IL-6 and acute phase proteins [Staels et al. 1998].

Studies of PPARγ in rat vascular SMC have shown that troglitazone inhibits the degradation of inducible NO synthase (iNOS) mRNA in a PPARγ-independent manner [Hattori et al. 1999]. In contrast, in aortic SMC expressing PPARγ, the PPARγ activators, PGJ2 and 9-HODE, induce type II-secreted phospholipase A2 (type II sPLA2) expression [Couturier et al. 1999]. Because type II sPLA2 increases lipoxygenase-induced HODE production [Neuzi et al. 1998], it has been postulated that type II sPLA2-induced HODE synthesis may constitute a positive autocrine loop in the aorta during inflammatory states. Troglitazone has also been shown to inhibit, in vascular SMC, the induction of c-fos and serum response element-induced transcriptional activation [Law et al. 1996]. Finally, PPARγ activation has been reported to inhibit PDGF-BB-induced SMC migration [Marx et al. 1998].

Both PPARα and γ appear to have protective effects on the activity of vascular SMC. PPARα ligands may reduce the inflammatory response within the vessel wall by at least interfering with NF-κB signaling. PPARγ ligands may specifically inhibit SMC migration, by interfering with processes involved in degradation of the extracellular matrix.

I.III.3.3. Effect of PPARs on vascular monocytes/macrophages

It has been well-demonstrated that macrophages are the predominant inflammatory leukocytes in a number of chronic inflammatory diseases, including atherosclerosis [Ross 1999]. Chinetti et al. [1998] have shown that PPARα and PPARγ are both expressed in differentiated human macrophages **(Fig. 5)**.

Although PPARγ has been postulated to promote monocyte-macrophage differentiation and to favor the uptake of oxLDL by macrophages [Tontonoz et al. 1998, Nagy et al. 1998], both natural and synthetic PPARγ have been found to inhibit the transcriptional activity of iNOS, MMP-9 and scavenger receptor A genes by interfering with AP-1, NF-κB and STAT-1 transcription factors [Ricote et al 1998]. Jiang et al. [1998] have also shown that incubation of human monocytes with the natural PPARγ ligand PG-J2 or synthetic agonists inhibits, at the transcriptional level, the production of several pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6. The most pronounced effects are observed with PG-J2, which may not be very selective for PPARγ and may also act through PPAR-independent mechanisms [Spiegelman et al. 1998, Petrova et al. 1999]. Recently evidence has been provided that PG-J2 may exert anti-inflammatory activities through the inhibition of IκB kinase [Castrillo et al. 2000, Rossi et al. 2000]. In contrast, the findings that the natural PPARα ligands LTB4 and 8(S)-HETE stimulate nitrite accumulation in RAW 264.7 murine macrophages suggest that these compounds may act as pro-inflammatory molecules [Colville-Nash et al. 1998].

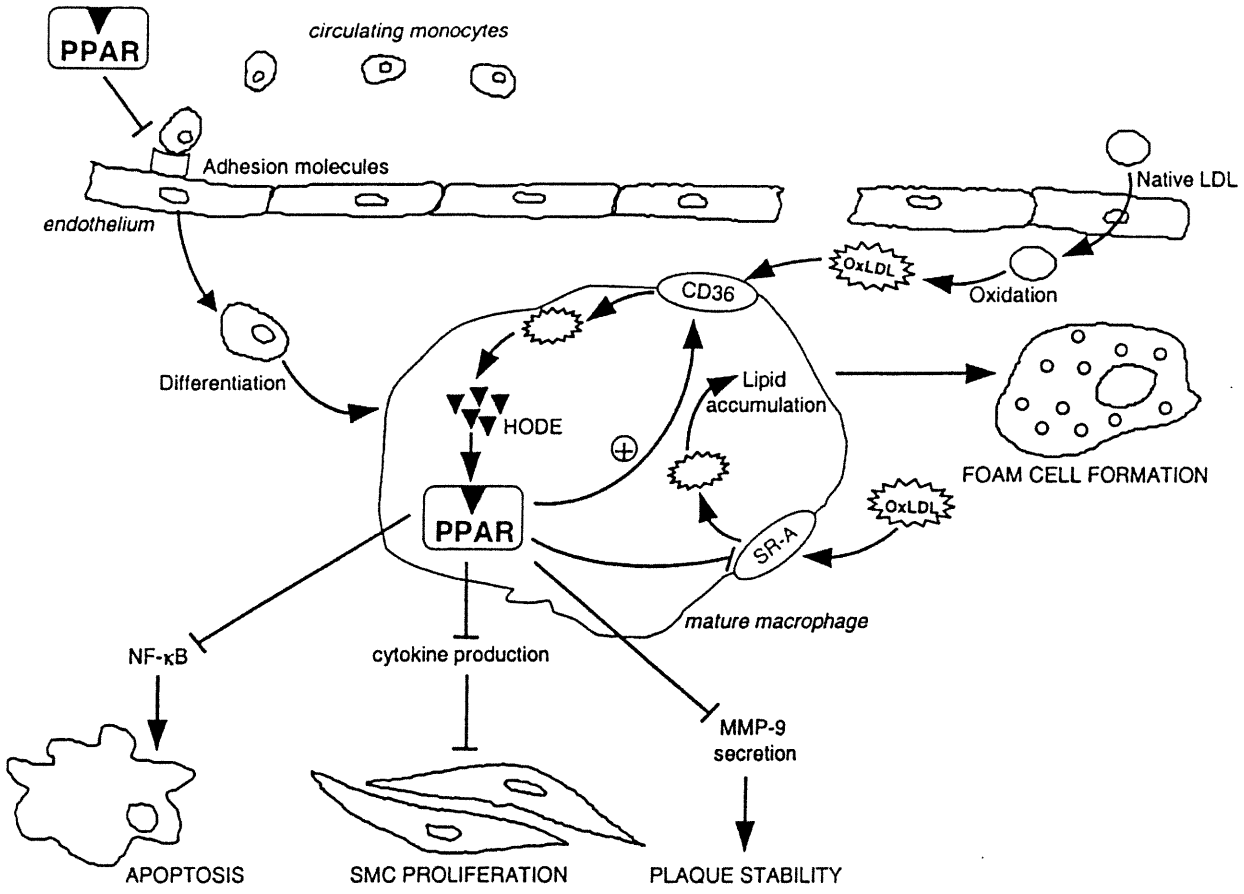


Fig. 5. PPARs control macrophage functions during atherosclerosis development [G. Chinetti et al. 2000]. PPAR activation controls the expression of genes implicated in monocyte adhesion, lipid accumulation (such as CD36 and SR-A), apoptosis, vascular inflammation (cytokines) and plaque stability (MMP-9), thus modulating atherosclerotic lesion development.

II. ARTICLE

Peroxisome proliferator-activated receptor α and γ agonists upregulate human macrophage lipoprotein lipase expression.

Ling Li [1], Marie-Claude Beauchamp[2] and Geneviève Renier[2]

From the CHUM Research Centre, Notre-Dame Hospital, Department of Biomedical Sciences [1] and Nutrition [2], University of Montreal, Montreal, Quebec, Canada.

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Reprint requests to:
Dr. Geneviève Renier
CHUM Research Centre
J.-A. De Seve Pavilion, Door Y 3622
Notre-Dame Hospital
1560 Sherbrooke Street East
Montreal, Quebec, H2L 4M1
E-MAIL: genevieve.renierg@umontreal.ca

ABSTRACT

Peroxisome proliferator-activated receptors (PPARs) are transcriptional factors which mediate pleiotropic effects including regulation of genes involved in lipid metabolism and control of inflammation. In the present study, we measured the in vitro effects of PPARα and γ ligands on macrophage lipoprotein lipase (LPL) expression. Human monocyte-derived macrophages (MDM) were cultured for 1 to 3 days in the presence of PPARα and γ ligands. At the end of these incubation periods, extracellular LPL immunoreactive mass/activity and LPL mRNA levels were measured. Incubation of human MDM with PPARα and γ ligands stimulated, in a time- and dose-dependent manner, human MDM LPL mass and activity. These agents also significantly increased macrophage LPL mRNA expression. In THP-1 cells treated with PPARα and γ ligands enhanced nuclear protein binding to the peroxisome proliferator responsive element (PPRE) of the human LPL promoter was observed. Furthermore, in these cells, a decreased rate of decay of LPL mRNA was documented. Overall, these results demonstrate that PPARα and γ activators increase macrophage LPL secretion. Given the proatherogenic effect of macrophage LPL in the vascular wall, better understanding of the biological role of PPARs in the regulation of macrophage LPL expression could lead to the development of new approaches in the prevention and treatment of atherosclerosis.

Keywords: Peroxisome proliferator-activated receptors, macrophage, lipoprotein lipase.

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of the superfamily of nuclear hormone receptors.[1-3] Members of this family include PPARα, PPARβ, and PPARγ. While PPARα is expressed preferentially in the liver and is an important regulator of lipid metabolism,[4-5] PPARγ is adipose tissue specific and plays a key role in adipocyte differentiation and glucose homeostasis.[6-7] PPARs function as ligand-dependent transcription factors, which upon heterodimerization with the 9-cis-retinoic acid receptor, bind to a specific response element termed peroxisome proliferator responsive element (PPRE) in the promoter of various genes. Several agents, including fatty acids, fibrates, eicosanoids and synthetic thiazolidinedione drugs activate PPARs.

It has been recently suggested that PPARs may play a key role in the pathogenesis of atherosclerosis. Indeed, it has been reported that principal participants in the atherosclerotic process namely monocytes/macrophages and macrophage-derived foam cells express PPARs. [8-11] Recent findings showing that PPARα and γ regulate genes implicated in the inflammatory response and modulate the differentiation process of macrophages into foam cells further support this possibility.[9, 12-13]

Macrophages present in the atherosclerotic vessel secrete lipoprotein lipase (LPL), a key enzyme in the metabolism of triglyceride-rich lipoproteins.[14-15] Arguments that point to macrophage LPL as a principal participant in atherogenesis include its ability to promote lipid accumulation within lesion macrophages, to induce monocyte/macrophage activation, to enhance monocyte binding to the endothelium and to stimulate vascular smooth muscle cell growth.[16-23] Despite evidence showing that PPAR agonists induce the transcriptional activation of the LPL gene through a PPRE located in the promoter of the LPL gene, [24-25]

the effect of PPAR ligands on macrophage LPL expression has not been studied yet. Given the potential key role of PPARs in atherogenesis, we investigated in the present study the effect of PPARα and γ ligands on human macrophage LPL gene expression, secretion and activity.

MATERIALS AND METHODS

Reagents

Fetal calf serum (FCS) was purchased from Hyclone Laboratories. RPMI 1640 medium, Hank's balanced salt solution, lymphoprep, penicillin-streptomycin, phorbol 12-myristate 13-acetate (PMA), glycine, sodium dodecyl sulfate (SDS) and TRIzol reagent were obtained from Gibco BRL (Burlington, Ontario, Canada). WY-14643, eisosatetraynoic acid (ETYA) and clofibrate were provided by Biomol. BRL-49653 and troglitazone were kindly provided by Parke Davis. Prostaglandin J_2 (PGJ_2) and GI-251929X were obtained from Calbiochem and Glaxo Wellcome, respectively. Formaldehyde solution was obtained from Fischer (Fair Lawn, NJ). [^{32}P] dCTP (specific activity 3,000 Ci/mmol) was provided by ICN Biochemicals (Costa Mesa CA).

Human macrophages

Human monocytes were isolated as previously described. [26] Briefly, peripheral blood mononuclear cells were isolated from healthy control subjects by density centrifugation using Ficoll, allowed to aggregate in the presence of FCS, then further purified by the rosetting technique. After density centrifugation, highly purified monocytes (85-90%) were recovered, as assessed by flow cytometry (FACScan, Becton Dickinson). Differentiation of monocytes into macrophages was achieved by culturing the freshly isolated monocytes ($2x10^6$/ml) in RPMI 1640 medium containing 20% (vol/vol) autologous serum for 8 days. The cells were incubated at 37°C in a humidified 5% CO_2, 95% air atmosphere. The culture medium was changed at days four and eight.

Human monocytic THP-1 cells were obtained from American Type Culture Collection and cultured in RPMI 1640 medium supplemented with 25 mmol/L HEPES buffer, 10% (vol/vol) FCS and 1% (vol/vol) penicillin-streptomycin (Flow, McLean, VA). THP-1 monocytes were differentiated into macrophages by treatment with 1 μmol/L PMA for 2 days.

THP-1 differentiated macrophages and human monocyte-derived macrophages (MDM) were treated for 1 to 3 days with PPARα (WY-14643, ETYA, clofibrate) or PPARγ ligands (PGJ_2, BRL-49653, troglitazone, and GI-251929X). Culture media containing DMSO or methyl acetate were used as controls.

Analysis of LPL mRNA expression

-Northern Blot Analysis:

$7X10^6$ THP-1-derived macrophages were plated in plastic petri dishes (100x20mm) (Falcon). Following treatment with appropriate agents, macrophages were lysed with guanidine isothiocyanate. Total RNA was purified by centrifugation through a cesium chloride gradient.[27] Twenty-five μg of total RNA were separated on a 1.2 % agarose gel containing 2.2 mol/L formaldehyde.[28] The blots were prehybridized for 8h with prehybridization buffer. The mRNA expression was analyzed by hybridization with [^{32}P] dCTP-labeled human LPL and GAPDH cDNA probes. Hybridization was detected by autoradiography. mRNA expression was quantified by high-resolution optical densitometry.

-Polymerase Chain Reaction:

Expression of the LPL gene in human MDM was performed by the polymerase chain reaction (PCR) technique. Total RNA for use in the PCR reaction was extracted

from $2x10^6$ human MDM by an improvement of the acid-phenol technique of Chomczynski.[29] Briefly, cells were lysed with TRIzol reagent and chloroform was added to the solution. After centrifugation, the RNA present in the aqueous phase was precipitated and resuspended in diethyl pyrocarbonate water. cDNA was synthesized from RNA by incubating total cellular RNA with 0.1 µg oligodT (Pharmacia) for 5 min at 98°C then by incubating the mixture with reverse transcription buffer for 60 min at 37°C. The cDNA obtained was amplified by using 0.8 µmol/L of two synthetic primers specific for human LPL (5'-GAGATTTCTCTGTATGGCACC-3') (5'-CTGCAAATGAGACACTTTCTC-3') and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5'-CCCTTCATTGACCTCAACTACATGG-3') (5'-AGTCTTCTGGGTGGCAGTGATGG-3'), used as internal standard in the PCR reaction mixture. A 277-base pair human LPL cDNA fragment and a 456-base pair human GAPDH cDNA fragment were amplified enzymatically by 22 and 25 repeated cycles, respectively. An aliquot of each reaction mixture was then subjected to electrophoresis on 1% agarose gel containing ethidium bromide. The intensity of the bands was measured by an image analysis scanning system (Alpha Imager 2000, Packard Instrument Company).

Electrophoretic mobility shift assay.

The isolation of the nuclei was performed as previously described.[30] Briefly, 5 x 10^7 THP-1 MDM were collected, washed with cold phosphate-buffered salt solution (PBS), and lysed in 1 ml ice-cold buffer A (15 nmol/L KCL, 2 mmol/L $MgCl_2$, 10 mmol/L HEPES, 0.1 % phenylmethylsulfonyl fluoride (PMSF), and 0.5% Nonidet-P-40). After a 10-min incubation on ice, lysed cells were centrifuged, and the nuclei were washed with buffer A without Nonidet P-40. The nuclei were then lysed in a buffer containing 2 mol/L KCL, 25 mmol/L HEPES, 0.1 mmol/L EDTA, and 1 mmol/L dithiothreitol (DTT). After a 15-min incubation period, a dialysis buffer (25 mmol/L HEPES, 1 mmol/L DTT, 0.1 % PMSF, 2 µg/ml aprotinin, 0.1 mmol/L EDTA, and 11% glycerol) was added to the nuclei

preparation. Nuclei were collected by centrifugation for 20 min at 13,000 rpm. Aliquots (50 μl) of the supernatants were frozen at -70°C, and protein concentration was determined. DNA retardation (mobility shift) electrophoresis assays were performed as previously described by Fried and Crothers.[31] Briefly, 5 μg nuclear extracts were incubated for 15 min in the presence of 5 X binding buffer (125 mmol/L HEPES, pH 7.5, 50 % glycerol, 250 mmol/L NaCl, 0.25 % Nonidet P-40, and 5 mmol/L DTT). End-labeled double-stranded consensus sequences of the LPL promoter PPAR-enhancing element (20 000 cpm per sample) were then added to the samples for 30 min. Samples were analyzed on a 4 % nondenaturing PAGE containing 0.01% Nonidet P-40. The specificity of the nuclear protein binding was assessed by incubating the nuclear proteins isolated from THP-1 macrophages with a labeled DNA probe in the presence of a 1,000-fold molar excess of unlabeled DNA probe.

DNA probes

The cDNA probes for detection of human LPL were prepared by the polymerase chain reaction technique. cDNA was obtained from total RNA using a reverse transcription reaction. Two synthetic primers spanning bases 255-287 and 1117-1149 of the LPL cDNA were used to enzymatically amplify a 894-bp region of the LPL probe. The cDNA probes for GAPDH were purchased from American Type Culture Collection. A 20-mer double-stranded oligonucleotide containing the peroxisome proliferator-responsive element (PPRE) consensus sequence of the human LPL gene promoter [24] (sense : 5'-CGTCTGCCCTTTCCCCCTCT-3'; antisense : 5'-GAGAAGAGGGGGAAAGG-3') was synthesized with the aid of an automated DNA synthesizer. After annealing, the double-

stranded oligonucleotide was labeled with [γ-^{32}P] ATP using the Boehringer-Mannheim 5' end-labeling kit (Indianapolis, IN).

Determination of LPL immunoreactive mass and activity

2X10^6 human MDM and THP-1 macrophages were cultured for 1 to 3 days with PPARα or γ ligands. One hour before the end of the incubation period, heparin (100 U/ml) was added to the medium. The amount of LPL immunoreactive mass in the supernatant was measured by enzyme-linked immunosorbent assay (ELISA) using the Markit-F-LPL kit. [32] Extracellular LPL activity was determined in the supernatants using the Confluolip kit.[33] Levels of LPL immunoreactive mass and activity were normalized to total cell protein levels and expressed as percentages of basal values.

Determination of total protein concentrations

Total protein content was estimated according to the Bradford method [34] by using a colorimetric assay (Bio-Rad, Mississauga, Ontario, Canada), and bovine serum albumin as standard.

Determination of cell viability

Cell viability following treatment with the PPAR agonists was assessed by trypan blue exclusion. Although cell viability was consistently found to be higher than 85%, a decrease in this parameter was observed in macrophages treated with high concentrations (1 μmol/L) of troglitazone.

Statistical Analysis

All values were expressed as the mean ± SEM to the mean. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test or the Dunn test. A P value less than 0.05 was considered statistically significant.

RESULTS

Effect of PPARα and γ agonists on LPL secretion by human MDM.

To assess the effects of activators of PPARs on human macrophage LPL secretion, human MDM were incubated for 1 to 3 days in the presence of both natural and synthetic PPARα and γ ligands. Incubation of human MDM with PPARα (WY-14643 (10 μmol/L), ETYA (20 μM), clofibrate (100 μmol/L)) or PPARγ agonists (PGJ_2 (1 μmol/L), BRL-49653 (20 nmol/L), troglitazone (0.01 μmol/L), GI-251929X (1 μmol/L)) led to a time-dependent increase in LPL mass and activity produced by these cells. Significant stimulation of LPL secretion into the medium was observed after a 2-day incubation period. Levels of MDM LPL mass and activity in response to PPARα and γ agonists are illustrated in Fig.1A and B and Fig.1C and D, respectively.

Dose-dependent effects of PPARα and γ agonists on LPL secretion by human MDM.

To identify the optimal concentration of PPARα and γ agonists required to stimulate macrophage LPL secretion, human MDM were cultured with various concentrations of PPARα and γ agonists for 2 days. As shown in Fig 2, PPARα and γ agonists increased extracellular LPL immunoreactive mass and activity in a dose-dependent manner. The levels of LPL mass and activity in response to increasing concentrations of WY (1-10 μmol/L) and clofibrate (10-100 μmol/L), are illustrated in Fig.2A and B. Both agents, at a concentration of 10μmol/L, exerted similar and maximal stimulatory effect on LPL mass. In contrast, maximal effects of WY and clofibrate on

LPL activity occurred at 1 μmol/L and 100 μmol/L, respectively. PPARγ agonists also stimulate in a dose-dependent manner the secretion of LPL mass and activity into the culture medium (Fig.2C and D). Maximal effect of troglitazone and GI on LPL mass and activity occurred at 1 μmol/L and 10 μmol/L, respectively.

Effect of PPARα and γ ligands on human MDM LPL mRNA expression.

To determine the effects of PPAR agonists on human MDM LPL gene expression, PCR analysis was performed. Our results demonstrated a sustained increase in LPL mRNA expression following treatment of human MDM for 1 to 3 days with both PPARα and γ ligands (Fig.3A and B). The effect of PPAR ligands on LPL gene expression was specific as reflected by the lack of modulation of the mRNA expression of the housekeeping gene GAPDH (Fig.3A and B). LPL mRNA levels, normalized to the levels of GAPDH mRNA are presented in Fig. 3A and B.

Molecular mechanisms involved in the stimulatory effect of PPARα and γ ligands on macrophage LPL gene expression.

On the basis of our observation that PPAR agonists upregulate human MDM LPL gene expression, we next determined whether incubation of macrophages in the presence of PPARα and γ agonists might induce changes at the level of the LPL gene promoter binding PPAR protein and LPL mRNA half-life. As part of a setup for subsequent gel-shift and mRNA stability data, LPL gene expression and protein levels in human MDM THP-1 cells exposed to these ligands were first determined.

Incubation of THP-1 macrophages with PPARα ligands for 2 days increased LPL mRNA levels (Fig.4A). A similar effect was observed when these cells were treated with PPARγ agonists (Fig.4D). In THP-1 cells exposed to PPARα and γ agonists, a significant increase in extracellular LPL mass (Fig.4B and E) and activity (Fig.4C and F) was also observed.

Incubation of THP-1 macrophages with clofibrate and GI increased the binding of nuclear proteins to the PPRE consensus sequence of the LPL promoter (Fig. 5A). This binding was specifically competed in the presence of a 1,000-fold molar excess of the unlabeled PPRE oligonucleotide (Fig, 5A).

To investigate the possibility that PPARs agonists might affect the mRNA half-life of macrophage LPL, the half-life of LPL mRNAs was measured in THP-1 cells treated with clofibrate and GI after the addition of D-actinomycin. As shown in Fig. 5B, decreased rate of decay of LPL mRNA was observed in both clofibrate- and GI-treated cells.

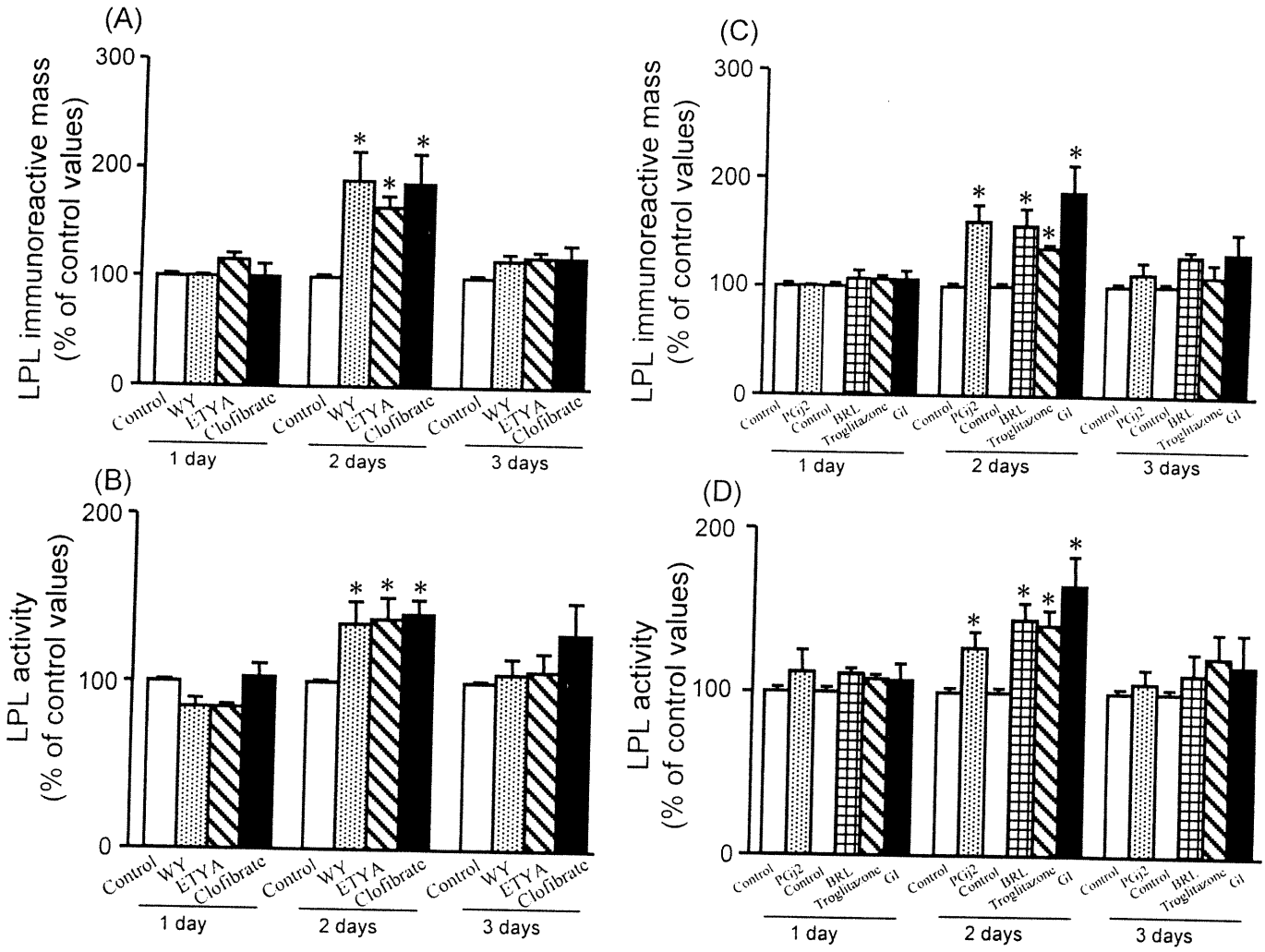

Figure.1. Effect of PPARα and γ ligands on LPL immunoreactive mass and activity in human MDM. Human MDM were cultured for 1 to 3 days in presence of PPARα ligands (WY-14643 (20 μmol/L), ETYA (10 μmol/L), clofibrate (100 μmol/L) or PPARγ ligands (PGJ_2 (1 μmol/L), BRL-49653 (20 nmol/L), troglitazone (0.01/μmol/L), GI-251929X (1 μmol/L)). At the end of the incubation periods, LPL immunoreactive mass (panels A, C) and activity (Panels B, D) were determined in the culture medium. Data represent the mean ± SEM of 4 experiments, *,$P<0.05$.

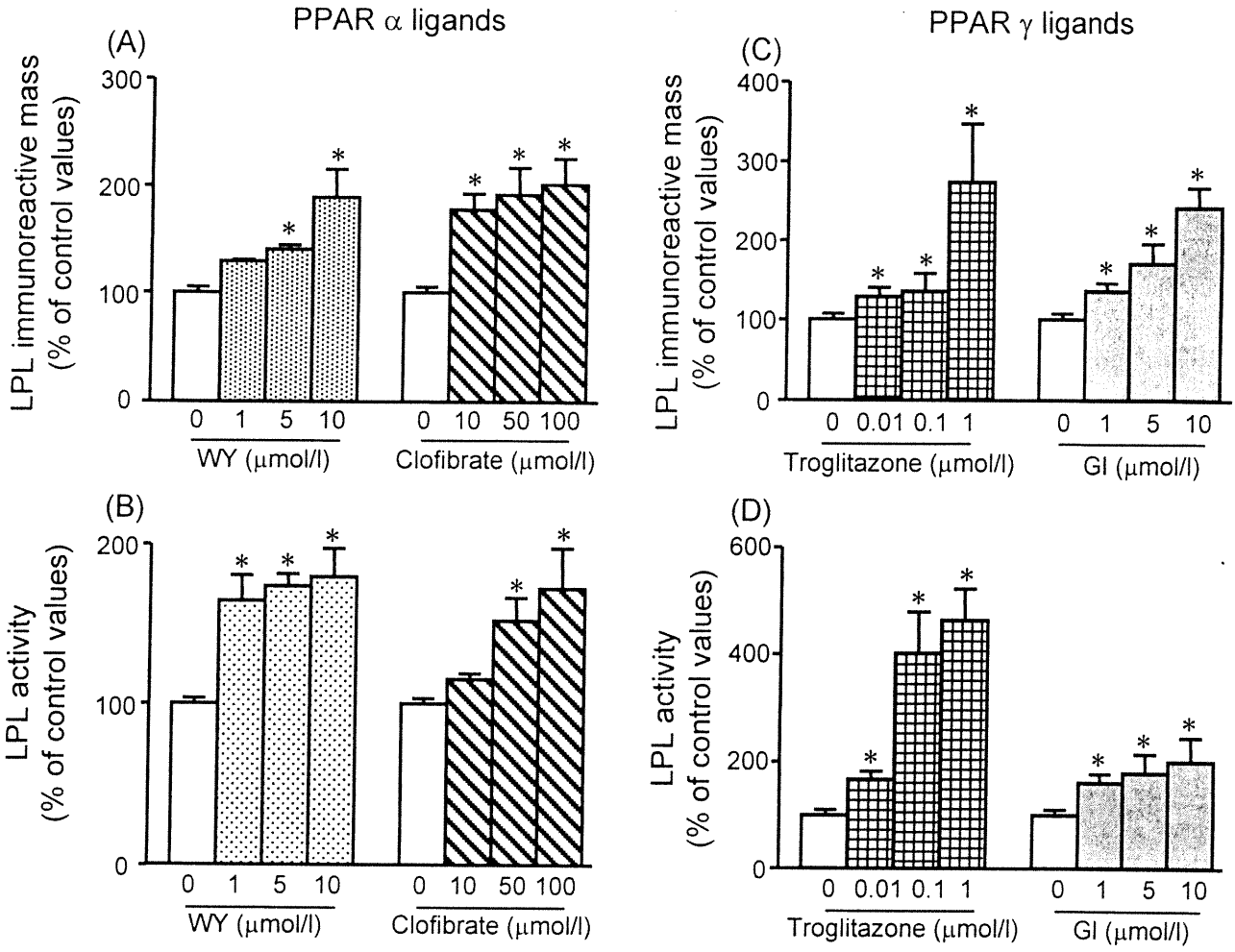

Figure.2. Dose-dependent effect of PPARα and γ ligands on human MDM LPL immunoreactive mass and activity. Human MDM were treated for 2 days with increasing concentrations of PPARα ligands or PPARγ ligands. At the end of the incubation period, LPL immunoreactive mass (Panels A, C) and activity (Panels B, D) were determined in the culture medium. Data represent the mean ± SEM of 5 experiments, *,P<0.05.

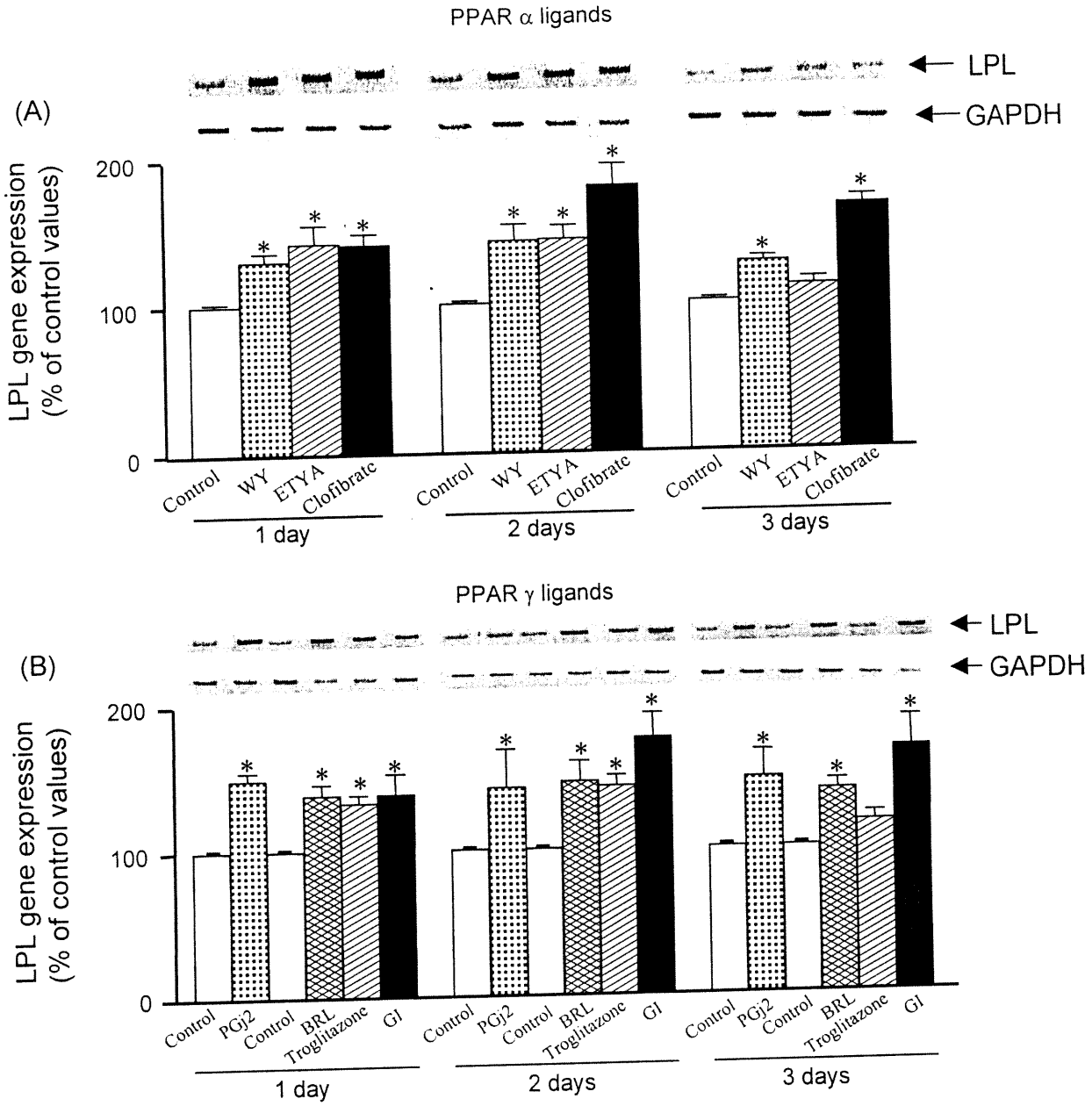
PPAR α ligands
(A)
LPL
GAPDH
LPL gene expression
(% of control values)
200
100
0
Control
WY
ETYA
Clofibrate
1 day
2 days
3 days
PPAR γ ligands
(B)
LPL
GAPDH
LPL gene expression
(% of control values)
200
100
0
Control
PGj2
Control
BRL
Troglitazone
GI
1 day
2 days
3 days

Figure.3. Effect of PPARα and γ ligands on human MDM LPL mRNA levels. Human MDM were cultured for 1 to 3 days in presence of PPARα ligands (Panel A) (WY-14643 (20 µmol/L), ETYA (10 µmol/L), clofibrate (100 µmol/L) or PPARγ ligands (Panel B) (PGJ_2 (1 µmol/L), BRL-49653 (20 nmol/L), troglitazone (0.01 µmol/L), GI-251929X (1 µmol/L)). At the end of the incubation periods, human MDM were lysed. LPL and GAPDH mRNA expressions were analyzed by RT-PCR. Graphs represent the levels of LPL mRNA normalized to the levels of GAPDH mRNA. Data represent the mean ± SEM of 5 experiments, *,$P<0.05$.

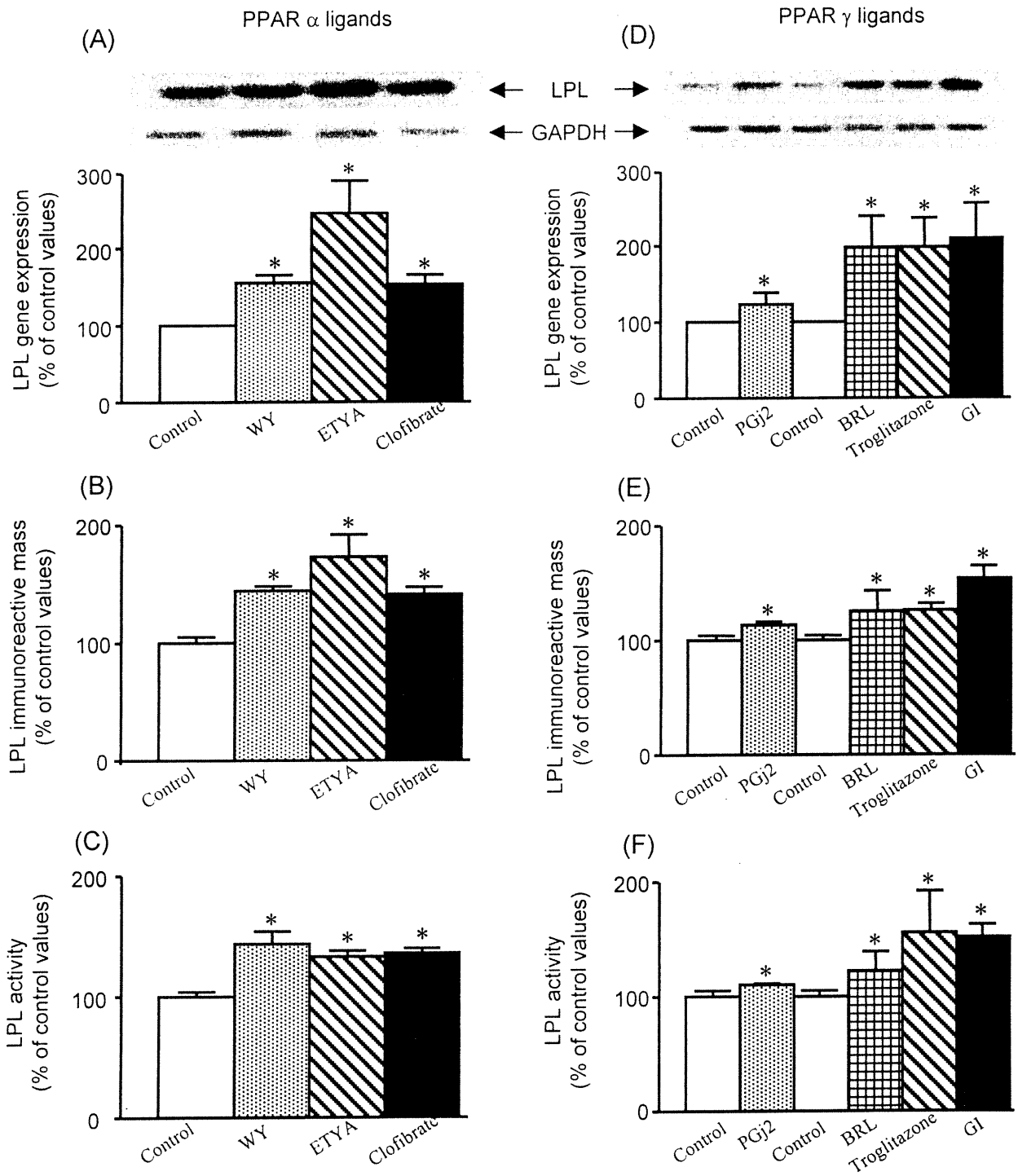
PPAR α ligands
PPAR γ ligands
(A)
(D)
LPL
GAPDH
LPL gene expression (% of control values)
300
200
100
0
Control
WY
ETYA
Clofibrate
PGj2
BRL
Troglitazone
GI
(B)
(E)
LPL immunoreactive mass (% of control values)
(C)
(F)
LPL activity (% of control values)

Figure. 4. Effect of PPARα and γ ligands on LPL mRNA levels , mass and activity in THP-1 macrophages. THP-1 macrophages were treated for 2 days with PPARα ligands (Panels A-C) (WY-14643 (20 µmol/L), ETYA (10 µmol/L), clofibrate (100 µmol/L)) or PPARγ ligands (Panels D-F) (PGJ_2 (1 µmol/L), BRL-49653 (20 nmol/L), troglitazone (0.01µmol/L), GI-251929X (1 µmol/L)). At the end of this incubation period, THP-1 macrophages were lysed. LPL and GAPDH mRNA expressions were analyzed by Northern blot analysis (Panels A,D). LPL immunoreactive mass (Panels B, E) and activity (Panels C, F) were determined in the culture medium. Data represent the mean ± SEM of 5 experiments, *,$P<0.05$.

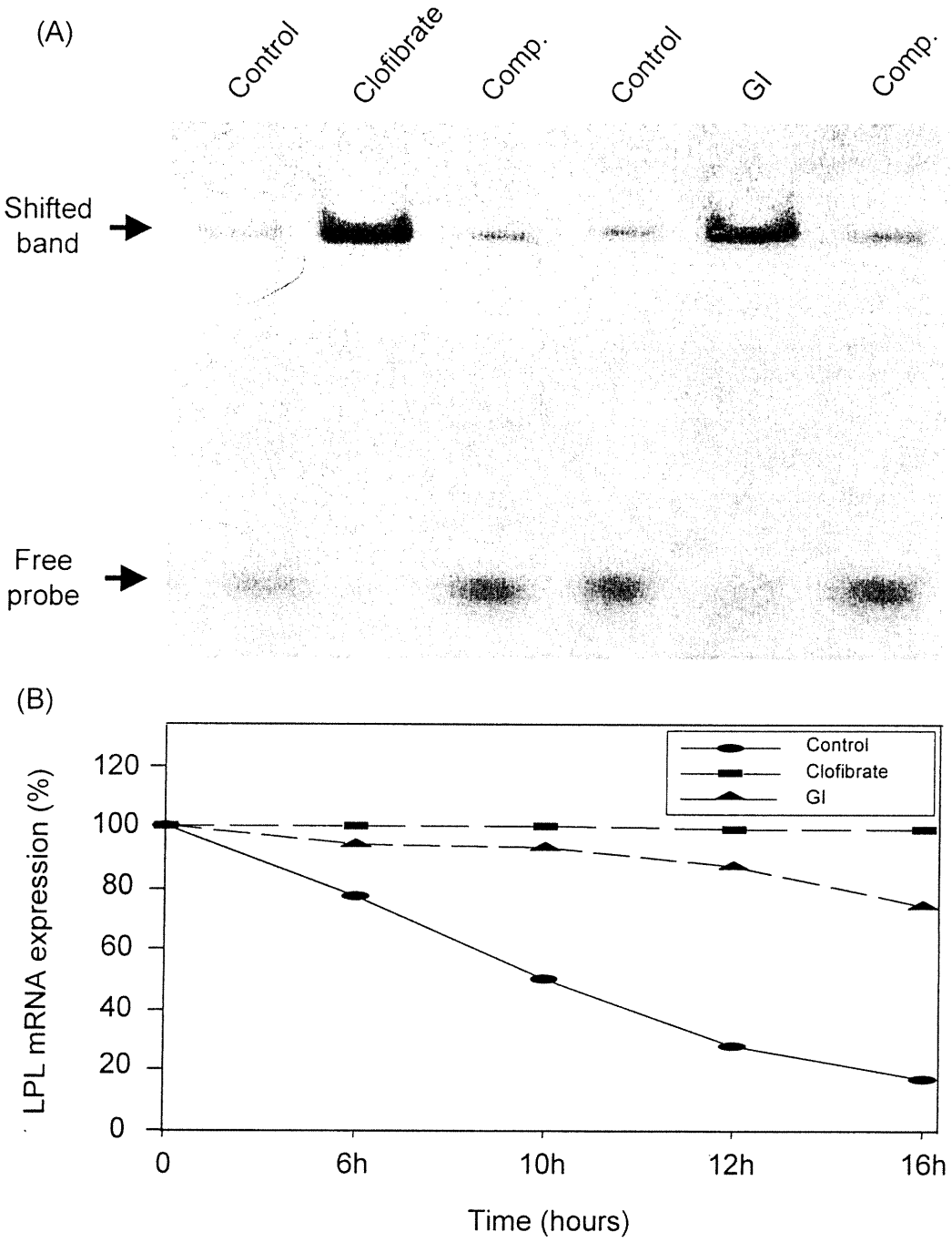
(A)
Control
Clofibrate
Comp.
Control
GI
Comp.
Shifted band
Free probe
(B)
Control
Clofibrate
GI
LPL mRNA expression (%)
120
100
80
60
40
20
0
0
6h
10h
12h
16h
Time (hours)

Figure. 5. Panel A: Effect of PPARα and γ ligands on the binding of nuclear proteins to the regulatory PPRE sequence of the LPL gene promoter (A). THP-1 macrophages were cultured for 2 days in the presence of clofibrate (100 μM) or GI (1 μM). At the end of this incubation period, nuclear proteins were isolated from the cells and incubated with end-labeled double-stranded consensus sequences of the LPL promoter PPAR-enhancing element. Retardation was assessed by gel electrophoresis in 4% PAGE. Data are the results of one representative experiment out of three.

Panel B: Effect of PPARα and γ ligands on the half-life of macrophage LPL mRNA. THP-1 macrophages were cultured for 2 days in the presence of clofibrate (100 μM) or GI (1 μM). Levels of LPL mRNA expression after 6, 10, 12, and 16 hours of actinomycin D treatment were calculated and plotted as a percentage expression compared with 100% of LPL mRNA extracted from cells exposed to these agents. Data represent the results of one representative experiment out of three.

DISCUSSION

The present study demonstrates for the first time that PPARα and γ ligands increase human macrophage LPL expression, both at the mRNA and protein level. Our data showing that PPAR agonists upregulate macrophage LPL mRNA levels identify unequivocally the macrophage LPL gene as a response gene for PPAR action. Previous observations have demonstrated that PPARα and γ activators induce LPL mRNA in liver and adipose tissue.[24-25] Inducibility of LPL by these compounds was correlated with the distribution of the respective PPAR isoforms: an adipocyte-restricted expression of PPARγ and a predominant expression of PPARα in liver. [24] Our findings that PPARα and γ ligands increase human MDM LPL mRNA expression is in accordance with previous results showing that human MDM express both PPARα and γ [5,35] and that well characterized PPARα and γ ligands, such as fatty acids, exert a direct modulatory effect on macrophage LPL gene expression. [36]

The regulatory effect of PPAR agonists on human macrophage LPL gene expression may theoretically involve both transcriptional and/or post-transcriptional mechanisms. Our data which show that PPARα and γ ligands increase the level of binding of nuclear proteins to a PPRE sequence element support the possibility that PPAR ligands may direct a transcriptional activation of the LPL gene via a PPRE sequence element. This possibility is supported by previous results demonstrating that a PPRE element present in the human LPL promoter mediates the functional responsiveness to fibrates and thiazolidinediones. [24] The hypothesis that PPAR ligands enhance the binding activity of the PPRE sequence in a PPAR-dependent manner awaits further studies aimed at evaluating the role of these agonists in LPL transcription

and reporter construct analysis. Alternatively, transcriptional activation of the macrophage LPL gene by PPAR agonists may occur through interference of activated PPARs with other transcription factor pathways, involved in LPL gene regulation such as stimulating protein-1. [37-38] Future studies are required to determine whether LPL transcriptional activation by PPAR agonists may involve this mechanism.

Determination of the levels of LPL mass/activity in the culture media of human MDM clearly demonstrates that PPARα and γ ligands lead to the production of increased amounts of catalytically active LPL. Although not very dramatic, the increased LPL secretion was observed with each of the agents used in the study. Because we tested a vast array of natural and synthetic PPAR ligands and used them at concentrations corresponding to their specific PPARα or γ binding affinities, our data suggest that induction of macrophage LPL secretion in response to these agents involves PPAR activation. However, because PPAR ligands have many PPAR-independent effects, [5,39-41] other mechanisms cannot be ruled out.

It is well known that LPL expression is controlled at both the transcriptional and post-transcriptional levels. Whereas adipocyte LPL regulation in response to PPARγ ligands has been proposed to take place mainly at the post-transcriptional level[42], our results suggest that regulation of macrophage LPL in response to PPAR agonists may involve both transcriptional and post-transcriptional events. The earliest point where post-transcriptional regulation can occur is at the level of LPL mRNA stability. Hattori et al recently demonstrated that troglitazone enhances nitric oxide synthase mRNA levels in vascular smooth muscle cells by prolonging its half-life.[43] In accordance with this observation, we found a decreased rate of decay of LPL mRNA in THP-1 cells exposed

to either PPARα or γ ligands. These data indicate that PPARα and γ ligands exert their stimulatory effect on macrophage LPL gene expression, at least partly, by enhancing LPL mRNA stability.

Data generated by time course and dose response experiments provide additional evidence that macrophage LPL expression in response to PPAR agonists may involve post-transcriptional events. One hand, results of time course experiments clearly indicate that induction of LPL secretion in response to PPAR agonists is transient despite a sustained increase of LPL mRNA levels from 1 to 3 days. This uncoupling between LPL gene expression and secretion suggests that regulation of macrophage LPL secretion in response to PPAR agonists occurs at the post-transcriptional level. On the other hand, treatment of human MDM with very low concentrations of PPAR agonists demonstrates increased secretion of inactive LPL but isolated activation of LPL activity in human MDM treated with clofibrate and WY, respectively. Such differential effect of PPAR activators on LPL mass and activity further suggest a regulation of macrophage LPL secretion in response to these agents at the post-translational level.

The discovery that PPARs are expressed in human atherosclerotic lesions and macrophage foam cells [8-11] has raised many questions about their role on atherogenesis. While evidence has been provided that PPAR agonists modulate vessel wall function [9-13,35,44-48], conflicting results have been generated about the role of PPARs in foam cell formation.[9, 49-50] Although the findings that PPARα and γ agonists lower the progression of atherosclerosis in humans and animals [51-53] suggest a net in vivo anti-atherogenic effect of PPAR activation, Tordjman et al recently demonstrated that high-

fat-fed PPARα-null mice develop less atherosclerosis.[54] Interestingly, these authors also documented higher levels of aortic mRNA for LPL in PPARα $^{+/+}$ vs in PPARα $^{-/-}$ mice. Based on the key role of macrophage LPL in atherosclerosis in vivo [55-58], results of this study raise the possibility that decreased arterial wall LPL expression may represent a mechanism for less atherosclerosis in PPARα $^{-/-}$ mice. These findings and our observations that PPARα ligands increase macrophage LPL secretion in vitro further stress the crucial role of these agents in the regulation of vascular wall LPL expression.

Resolving the question whether PPAR ligands favor or inhibit atherogenesis in humans is of major clinical interest, especially for diabetic patients who are at high risk of accelerated atherosclerosis and are treated with PPAR agonists. The relevance of this question is further emphasized by our recent findings that human type 2 diabetes is associated with altered macrophage PPAR gene expression[59] and increased LPL secretion.[60] Detailed understanding of the role of PPARs in the regulation of vascular LPL expression and development of novel PPAR ligands that dissociate pro- and antiatherogenic properties will offer new strategies in the treatment of atherosclerosis.

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REFERENCES

1. Schoonjans K, Martin G, Staels B, Auwerx J. Peroxisome proliferator-activated receptors, orphans with ligands and functions. *Curr Opin Lipidol.* 1997;8:159-166.
2. Desvergne B, Wahli W. PPAR: a key nuclear factor in nutrient/gene interactions? In Baeuerle PA, ed. *Inducible gene expression.* Boston, MA: Birkhäuser Publishers; 1995: 142-176.
3. Desvergne B, Ijpenberg A, Devchand PR, Wahli W. The peroxisome proliferator-activated receptors at the cross-road of diet and hormonal signalling. *J Steroid Biochem Mol Biol.* 1998;65:65-74.
4. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci USA* 1994;91:7355-7359.
5. Braissant O, Foufelle F, Scotto C, Dauca M, Whali W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology* 1996;137:354-366.
6. Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* 1994;135:798-800.
7. Tontonoz P, Hu E, Devine J, Beale EG, Spiegelman BM. PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol.* 1995:15:351-357.

8. Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, Hsieh L, Greene G, Nimer SD. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: Expression in hematopoietic cells and chromosomal mapping. *Gene Expr.*. 1995;4:281-299.

9. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* 1998;93:241-252.

10. Marx N, Sukhova G, Murphy C, Libby P, Plutzky J. Macrophages in human atheroma contain PPAR gamma: differentiation-dependent peroxisomal proliferator-activated receptor gamma (PPAR gamma) expression and reduction of MMP-9 activity through PPAR gamma activation in mononuclear phagocytes in vitro. *Am J Pathol.* 1998;153:17-23.

11. Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, Witztum JL, Auwerx J, Palinski W, Glass CK. Expression of the peroxisome proliferator-activated receptors (PPARs) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc Natl Acad Sci USA* 1998;95:7614-7619.

12. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* 1998;31:79-82.

13. Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998;31:82-86.

14. Eckel, RH. Lipoprotein lipase : a multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med.* 1989; 320, 1060-1068.

15. O'Brien KD, Gordon D, Deeb S, Ferguson M, Chait A. Lipoprotein lipase is synthesized by macrophage-derived foam cells in human coronary atherosclerotic plaques. *J Clin Invest.* 1992;89:1544-1550.

16. Lindqvist P, Ostlund-Linqvist AM, Witztum JL, Steinberg D, Little JA. The role of lipoprotein lipase in the metabolism of triglyceride-rich lipoproteins by macrophages. *J Biol Chem.* 1983; 258: 9086-9092.

17. Ostlund-Lindqvist AM, Gustafson S, Lindqvist P, Witztum JL, Little JA. Uptake and degradation of human chylomicrons by macrophages in culture : role of lipoprotein lipase. *Arteriosclerosis.* 1983; 3: 433-440.

18. Ishibashi S, Yamada N, Shimano H, Mori N, Mokuno H, Gotohda T, Kawakami M, Murase T, Takaku F. Apolipoprotein-E and lipoprotein lipase secreted from human monocyte-derived macrophages modulate very low density lipoprotein uptake. *J Biol Chem.* 1990; 265: 3040-3047.

19. Mamputu J-C, Desfaits AC, Renier G. Lipoprotein lipase enhances human monocyte adhesion to aortic endothelial cells. *J Lipid Res.* 1997; 38: 1722-1729.

20. Obunike JC, Paka S, Pillarisetti S, Goldberg IJ. Lipoprotein lipase can function as a monocyte adhesion protein. *Arterioscler Thromb Vasc Biol.* 1997; 17: 1414-1420.

21. Renier G, Skamene E, DeSanctis JB, Radzioch D. Induction of tumor necrosis factor alpha gene expression by lipoprotein lipase. *J Lipid Res.* 1994; 35 : 2271-2278.

22. Mamputu J-C, Levesque L, Renier G. Differentiation of human monocytes to monocyte-derived macrophages is associated with increased lipoprotein lipase-induced tumor necrosis factor-α expression and production. A process involving cell surface proteoglycans. *Arterioscler Thromb Vasc Biol.* 1999; 19 : 1405-1411.

23. Mamputu J-C, Renier G. Proliferative effect of lipoprotein lipase on human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 2000; 20 : 2212-2219.

24. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *Embo J.* 1996;15:5336-5348.

25. Auwerx J, Schoonjans K, Fruchart JC, Staels B. Transcriptional control of triglyceride metabolism: fibrates and fatty acids change the expression of the LPL and apo C-III genes by activating the nuclear receptor PPAR. *Atherosclerosis* 1996;124:S29-S37.

26. Mentzer SJ, Guyre PM, Burakoff SJ, Faller DV. Spontaneous aggregation as a mechanism for human monocyte purification. *Cell Immunol.* 1986;101:312-319.

27. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1979;18:5294-5299.

28. Thomas P. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc Natl Acad Sci USA* 1980;77:5201-5205.

29. Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987;162:156-159.

30. Han J, Beutler B, Huez G. Complex regulation of tumor necrosis factor mRNA turnover in lipopolysaccharide-activated macrophages. *Biochim Biophys Acta* 1991;1090:22-28.

31. Fried M, Crothers DM. Equilibria and kinetics of *lac* repressor-operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Res* 1981;9:6505-6525.

32. Ikeda Y, Takagi A, Ohkaru Y, Nogi K, Iwanaga T, Kurooka S, Yamamoto A. A sandwich-enzyme immunoassay for the quantification of lipoprotein lipase and hepatic triglyceride lipase in human postheparin plasma using monoclonal antibodies to the corresponding enzymes. *J Lipid Res.* 1990; 31:1911-1924.

33. Duque M, Graupner M, Stutz H, Wicher I, Zechner R, Paltauf F, Hermetter A. New fluorogenic triacylglycerol analogs as substrates for the determination of lipase activities. *J Lipid Res.* 1996;37:868-876.

34. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72: 248-254.

35. Chinetti G, Griglio S, Antonucci M, Pineda Torra I, Delerive P, Majd Z, Fruchart JC, Chapman J, Najib J, Staels B. Activation of peroxisome proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages. *J Biol Chem.* 1998;273 :25573-25580.

36. Michaud SE, Renier G. Direct regulatory effect of fatty acids on macrophage lipoprotein lipase : potential role of PPARs. *Diabetes* 2001;50:660-666.

37. Krey G, Mahfoudi A, Wahli W. Functional interactions of peroxisome proliferator-activated receptor, retinoid-X receptor, and Sp1 in the transcriptional regulation of the acyl-coenzyme-A oxidase promoter. *Mol Endocrinol.* 1995; 9:219-31.

38. Yang WS, Deeb SS. Sp1 and Sp3 transactivate the human lipoprotein lipase gene promoter through binding to a CT element: synergy with the sterol regulatory element binding protein and reduced transactivation of a naturally occurring promoter variant. *J Lipid Res.* 1998; 39:2054-64.

39. Fulgencio J-P, Kohl C, Girard G, Pegorier J-P. Troglitazone inhibits fatty acid oxidation and esterification, and gluconeogenesis in isolated hepatocytes from starved rats. *Diabetes* 1996;45 :1556-1562.

40. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre A, Saladin R, Najib J, Laville M, Fruchart J-C, Deeb S, Vidal-Puig V, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J. The organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol Chem.* 1997;272:18779-18789.

41. Cominacini L, Garbin U, Pastorino AM, Campagnola M, Fratta Pasini A, Davoli A, Rigoni A, Lo Cascio V. Effects of troglitazone on in vitro oxidation of LDL and HDL induced by copper ions and endothelial cells. *Diabetologia* 1997;40:165-172.

42. Ranganathan S, Kern P.A. Thiazolidinediones inhibit lipoprotein lipase activity in adipocytes. *J. Biol. Chem.* 1998, 40:26117-26122.

43. Hattori Y, Hattori S, Kasia K. Troglitazone upregulates nitric oxide synthesis in vascular smooth muscle cells. *Hypertension* 1999, 33 :943-948.

44. Deschand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The PPARα–leukotriene B_4 pathway to inflammation control. *Nature* 1996;384 :39-43.

45. Chinetti G, Gbaguidi FG, Griglio S, Mallat Z, Antonucci M, Poulain P, Chapman J, Fruchart JC, Tedgui A, Najib-Fruchart J, Staels B. CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. *Circulation* 2000;101:2411-2417.

46. Inoue H, Tanabe T, Umesono K. Feedback control of cyclooxygenase-2 expression through PPARγ. *J Biol Chem.* 2000;275:28028-28032.

47. Kintscher U, Goetze S, Wakino S, Kim S, Nagpal S, Chandraratna RAS, Graf K, Fleck E, Hsueh WA, Law RE. Peroxisome proliferator-activated receptor and retinoid X receptor ligands inhibit monocyte chemotactic protein-1-directed migration of monocytes. *Eur J Pharmacol.* 2000;401:259-270.

48. Shu H, Wong B, Zhou G, Li Y, Berger J, Woods JW, Wright SD, Cai T-Q . Activation of PPARα or γ reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells. *Biochem Biophys Res Comm.* 2000;267:345-349.

49. Moore KJ, Rosen ED, Fitzgerald ML, Randow F, Andersson LP, Altshuler D, Milstone DS, Mortensen RM, Spiegelman BM, Freeman MW. The role of PPAR-gamma in macrophage differentiation and cholesterol uptake. *Nat Med* 2001;7 :41-47.

50. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Pineda Torra I, Teissier E, Minnich A, Jaye M, Duverger N, Brewer HB, Fruchart JC, Clavey V, Staels B. PPAR-α and PPAR-γ activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat Med* 2001;7 :53-58.

51. Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, Faas FH, Linares E, Schaefer EJ, Schectman G, Wilt TJ, Wittes J. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. *N Engl J Med* 1999;341 :410-418.

52. Chen Z, Ishibashi S, Perrey S, Osuga Ji, Gotoda T, Kitamine T, Tamura Y, Okazaki H, Yahagi N, Iizuka Y, Shionoiri F, Ohashi K, Harada K, Shimano H, Nagai R, Yamada N. Troglitazone inhibits atherosclerosis in apolipoprotein E-knockout mice : pleiotropic effects on CD36 expression and HDL. *Arterioscler Thromb Vasc Biol.* 2001;21:372-377.

53. Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass W. Peroxisome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J Clin Invest* 2000;106 :523-531.

54. Tordjman K, Bernal-Mizrachi C, Zemany L, Weng S, Feng C, Zhang F, Leone TC, Coleman T, Kelly DP, Semenkovich CF. PPARα deficiency reduces insulin resistance and atherosclerosis in apoE-null mice. *J. Clin Invest* 2001;107 :1025-1034.

55. Semenkovich CF, Coleman T, Daugherty A. Effects of heterozygous lipoprotein lipase deficiency on diet-induced atherosclerosis in mice. *J Lipid Res.* 1998;39: 1141-1151.

56. Babaev VR, Fazio S, Gleaves LA, Carter KJ, Semenkovich CF, Linton MF. Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in vivo. *J Clin Invest.* 1999;103:1697-1705.

57. Babaev VR, Patel MB, Semenkovich CF, Fazio S, Linton MF. Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in LDL receptor deficient mice. *J Biol Chem.* 2000;275 :26293-26299.

58. Van Eck M, Zimmermann R, Groot PHE, Zechner R, Van Berkel TJC. Role of macrophage-derived lipoprotein lipase in lipoprotein metabolism and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2000;20:53-62.

59. Sartippour MR, Renier G. Differential regulation of macrophage peroxisome proliferator-activated receptor expression by glucose: role of peroxisome proliferator-activated receptors in lipoprotein lipase gene expression. *Arterioscler Thromb Vasc Biol.* 2000; 20 :104-10.

60. Sartippour MR, Renier G. Upregulation of macrophage lipoprotein lipase in patients with type 2 diabetes. *Diabetes* 2000;49:597-601.

III. **DISCUSSION**

Cardiovascular diseases are the main cause of morbidity and mortality in diabetes. The high prevalence of these complications in diabetes results from accelerated atherosclerosis in diabetic patients.

LPL, an enzyme known to undergo significant functional alterations in the diabetic state, acts as a proatherogenic protein in the vessel wall. Indeed multiple studies indicate that LPL produced by macrophages in the vascular wall favors the development of atherosclerosis by promoting lipid accumulation within atherosclerotic lesions [O'Brien et al. 1992, Yla-Herttuala et al. 1991, Mattsson et al. 1994, Van Eck et al. 2000, Santamarina-Fojo et al. 2000].

PPARs, like LPL, are key factors in atherogenesis [Fruchart et al. 1999, Sartippour et al. 2000, Plutzky et al. 2000]. PPARs are transcriptional factors that regulate the expression of several genes encoding proteins involved in lipid metabolism, including LPL. For example, PPARα activation increases apoAI and apoAII transcription and interferes with HDL-mediated reverse cholesterol transport [Vu-Dac et al. 1994, 1995]. This PPAR isoform also stimulates LPL expression [Schoonjans et al. 1996] and inhibits the expression of apoCIII [Staels et al. 1995]. PPARγ also regulates lipid metabolism and acts as a regulator of adipocyte differentiation [Fajas et al. 1998, Spiegelman et al. 1997, Rosen et al. 1999].

The present study investigated LPL gene expression in human and THP-1 macrophages in response to PPAR ligands. We showed that PPARα and PPARγ ligands directly enhanced macrophage gene expression in both THP-1 and human MDM. It is well known that the regulation of LPL gene expression is controlled by several *cis*- and *trans*-acting factors, such as PPRE, AP-1 and SP-1 regulatory elements, present in the LPL promoter [Enerback et al. 1993]. Schoonjans et al. [1996] have demonstrated that PPARα and PPARγ ligands induce in vivo LPL mRNA levels selectively in the liver and adipose tissue, respectively. PPARγ ligands have also been shown to increase LPL mRNA levels in adipocytes [Ranganathan and Kern, 1998].

Evidence has been provided that transcriptional activation of the LPL gene by PPARα and γ ligands is mediated by the interaction of PPAR-RXR heterodimers with PPRE in the LPL promoter [Schoonjans et al. 1996]. This possibility has been supported recently by Sartippour et al. [2000], who reported that anti-PPARα decreases glucose-induced enhancement of nuclear protein binding to the PPRE regulatory domain of the LPL gene. It has also been established that PPAR can repress gene transcription by a combination of protein-protein interaction and co-factor squelching without binding to PPRE. For example, promoter studies have revealed that PPARγ inhibits the transcriptional activity of iNOS, MMP-9 and scavenger receptor A genes by interfering with AP-1, NF-κB and STAT-1 transcription factors [Ricote et al. 1998]. Krey et al. [1995] also demonstrated that the PPAR/RXR heterodimer interacts with SP-1 on the native TATA-less acyl-coenzyme-A (ACO) promoter. Based on such findings,

one may postulate that PPAR ligands may interfere positively with these transcriptional factors to regulate LPL gene expression. Further studies are necessary to delineate which transcriptional factor is responsible for PPAR signaling on LPL gene expression.

The selective affinity of PPARα and PPARγ ligands for PPARs has been well-documented [Lin et al. 1999, Lehmann et al. 1995, Forman et al. 1995, Willson et al. 1996], but their effect on human macrophage LPL expression was investigated for the first time in our experiments. Our data demonstrated unequivocally that both PPARα and PPARγ ligands increase macrophage LPL gene expression.

Although our data showed a similar magnitude of LPL mRNA changes upon exposure of macrophages to PPARα or PPARγ ligands, we found a temporal difference of LPL mRNA expression in PPARα- and PPARγ-ligand-treated THP-1 MDM (data not shown). Indeed, our results demonstrated that in THP-1 MDM, PPARγ ligands induced LPL gene expression by day 1, whereas PPARα ligands heightened LPL gene expression by day 2. These results are consistent with those of Schoonjans et al. [1996] which demonstrate that the PPARγ ligand, BRL 49653, induces preadipocyte LPL mRNA levels much quicker than the PPARα ligand, fenofibrate [Schoonjans et al. 1996]. The mechanisms responsible for the slower kinetics of LPL mRNA induction after PPARα ligand treatment are unknown.

Our data also showed that the response of LPL gene expression to various PPAR agonists is not consistent. For example, among the PPARα

ligands, clofibrate is most potent in human MDM, but a maximal increase in THP-1 MDM is observed with ETYA. In addition, among the PPARγ agonists, the synthetic ligands, BRL, troglitazone, and GI are more potent than the natural ligand PGJ2 in inducing THP-1 MDM LPL gene expression, whereas similar responses are observed in response to all these ligands in human MDM. While species specificity in ligand recognition has been well documented between human and mouse, these results further suggest that specificity in ligand recognition may also occur in different human macrophage model systems. Whether specific characteristics of the transformed THP-1 macrophages are responsible for such differences is presently unknown.

Overall, our results demonstrate that both PPARα- and PPARγ-ligands are potent inducers of LPL mRNA levels in human macrophages. Because LPL expression is controlled at both the transcriptional and post-transcriptional levels, the amounts of LPL mass and activity secreted by human macrophages in response to PPAR α and γ agonists were next determined.

Several studies are consistent with LPL regulation in a tissue- and cell-specific manner [Eckel et al. 1989, Ding et al. 2000], notably in different macrophages [Behr et al. 1986, Hulten et al. 1996]. In 1983, Robinson et al. suggested that LPL could exist in the cell as an inactive precursor that required for its activation the presence of apoCII. Vannier et al. [1986] have shown that LPL is localized mainly in the Golgi, thereby establishing that the enzyme is a secretory protein. Today it is well established that LPL, in its active form, plays a central role in the overall lipid metabolism of the body. Specifically, the major role

of this enzyme in hydrolyzing TG-rich lipoproteins such as chylomicrons and VLDL and directing triglyceride-fatty acid traffic to fulfill the energy requirements of peripheral tissues is well documented [Robinson et al. 1983, Borensztajn et al. 1987, Ladu et al. 1991].

Both the hydrolytic and structural properties of LPL are involved in the regulatory effect of this enzyme on lipid metabolism. Indeed, LPL has been demonstrated to increase the cell association of lipoproteins by its bridging function [Goldberg et al. 1996] and recent experiments in transgenic mice expressing catalytically inactive LPL have provided evidence for LPL bridging in vivo [Merkel et al. 1998].

Alterations of LPL regulation have been associated with several disorders such as hypertriglyceridemia [Eckel et al. 1989] and atherogenesis [Babaev et al. 1999, 2000]. Because of the major role of LPL in both physiological and pathological states, there has been recently a great interest in studying the regulation of LPL (mass and activity) secretion by different cells and under various experimental conditions.

Relevantly, the present investigation demonstrates for the first time that both PPARα and PPARγ ligands increase the amount of extracellular LPL immunoreactive mass and activity in THP-1 and human MDM. Despite the fact that both cell types release more LPL in response to PPAR agonists, their response to these agents also differs in many ways: Firstly, the amount of LPL protein secreted upon exposure of these cells to PPAR ligands is significantly higher in human macrophages than in THP-1 MDM. In addition, in contrast to

human MDM, PPAR ligands only moderately enhance LPL mass and activity in THP-1 cells, thereby leading to the production of small amounts of catalytically active LPL. The response of LPL to the various PPAR ligands also differs between the THP-1 cells and human MDM. Among the PPARα ligands, ETYA is most potent in THP-1 MDM while a maximal increase in human MDM is observed with WY and clofibrate. Differential response of THP-1 and human MDM to PPAR agonists may be linked to preferential binding affinity of these compounds for either cell types. Alternatively, involvement of differential signal transduction pathways and/or molecular events may explain, at least partly, the specific response of these cells to the different compounds tested in our study. This hypothesis is supported by recent results showing the involvement of several signal transduction pathways, such as protein tyrosine phosphatase activity [Maegawa et al. 1995] and calcium fluxes [Buchanan et al. 1995] in the effect of PPAR ligands.

It is well-established that LPL gene expression is controlled at both the transcriptional and post-transcriptional levels. Our results which demonstrate that PPAR ligands induced parallel changes in macrophage LPL mRNA levels, mass and activity at day 2 suggest that macrophage LPL regulation in response to these agents occurs at least partly at the transcriptional level. While this possibility is supported by the finding that fibrates regulate apoCIII and LPL at the transcriptional level [Stael et al. 1992, 1995], it is in contrast with other results showing that treatment of preadipocytes with PPARγ ligands increase LPL mRNA levels but inhibit LPL activity [Ranganathan and Kern, 1998]. These results which

demonstrate differential regulation of macrophage and adipocyte LPL in response to PPAR agonists further stress the complex and cell-specific regulation of LPL. Our result also suggest that regulation of macrophage LPL in response to PPAR agonists may involve post-transcriptional events. Indeed, results of time course experiments clearly indicate that induction of LPL secretion in response to PPAR agonists is transient despite a sustained increase of LPL mRNA levels from 1 to 3 days. This uncoupling between LPL gene expression and secretion suggests that regulation of macrophage LPL secretion in response to PPAR agonists occurs at the post-transcriptional level. On the other hand, treatment of human MDM with very low concentrations of PPAR agonists demonstrate increased secretion of inactive LPL but isolated activation of LPL activity in human MDM treated with clofibrate and WY, respectively. Such differential effect of PPAR activators on LPL mass and activity further suggests a regulation of macrophage LPL secretion in response to these agents at the post-translational level.

PPAR ligands may induce macrophage LPL mRNA levels by altering mRNA stability. Previous studies have demonstrated that LPS and IFNγ decrease LPL mRNA stability [White et al. 1988, Jonasson etal. 1990]. Moreover, Ranganathan et al have demonstrated that adipocyte LPL translation is regulated by a catecholamine-activated protein that binds to the 3' untranslated region of the mRNA [Ranganathan et al. 1997]. Recently, Hattori et al [1999]. demonstrated that troglitazone enhances nitric oxide synthase mRNA levels in vascular smooth muscle cells by prolonging its half-life. To evaluate the

possibility that PPAR ligands may affect macrophage LPL gene expression by altering LPL mRNA stability, we measured the half-life of LPL mRNA in THP-1 MDM exposed to PPAR ligands. In accordance with this observation, we found a decreased rate of decay of LPL mRNA in THP-1 cells exposed to either PPARα or γ ligands. These data further establish that macrophage LPL is controlled both at the transcriptional and posttranscriptional levels by PPARs ligands.

It has been well documented that many PPAR agonists exhibit, when used at high concentrations, activity towards both PPARα and PPARγ. For example, ETYA which fully activates PPARα at a concentration of 1μM, with an ED 50 of 200nM, [Keller et al. 1993] also activates PPAR γ at high concentrations. Conversely, PGJ2 which is specific for PPARγ at low concentrations acts as a PPARα ligand at high concentrations. Examination of the ligand specificities of PPARs is complicated by the need to physically separate protein-bound and free ligands for measurement of equilibrium dissociation constants (K_d). Determination of the K_ds for the formation of PPAR ligand-receptor complexes indicates that the formation of ligand-protein complexes is in the range of 10-50 μM in vitro, which is higher than the ligand concentration of 5-60 nM in vivo [Lin et al. 1999]. The concentrations of the PPAR agonists used in our study have been chosen according to the estimated K_d of each compound. For example most of the thiazolidinediones have been found to have affinities for PPARγ between 30 nM (BRL49653) and 700 nM (troglitazone), while WY14643 exhibits high specificity for PPARα at a K_d value of 10 μM. It should be noted that normal therapeutic doses of troglitazone result in blood levels that exceed its K_d for the

receptor. Given that full and specific agonist activity on PPARα or PPARγ will ultimately depend on its concentrations, we chose to use these compounds at concentrations that do not exceed their K_ds. This careful experimental approach allows us to strongly suggest that the observed stimulatory effect of the PPAR agonists tested in our study on macrophage LPL expression are mediated through PPAR activation and to relate these effects to PPARα or γ activation. Dose response studies have also resulted in identification of optimal concentration of each ligand. Results of these studies demonstrate that both PPARα and γ ligands induce human macrophage LPL mass and activity in a dose-dependent manner. They also establish a differential stimulatory effect of low concentrations of PPARα ligands on LPL mass and activity, indicating a posttranslational control of this enzyme by these agents.

It has been well documented that the different PPAR isoforms are characterized by distinct tissue- and cell-specific expression patterns and exhibit distinct activation by various ligands. These observations suggest highly specific functions for each of these PPAR subtypes. While PPARα is mostly present in tissues characterized by high rates of fatty acid catabolism, such as the liver and plays a key role in lipid metabolism [Kliewer et al. 1994, Braissant et al. 1996], PPARγ is adipose tissue selective and is implicated as a mediator of adipocyte differentiation and regulation of glucose homeostasis [Chawla et al. 1994, Tontonoz et al. 1995].

Recent findings demonstrate that PPARs are also expressed in vascular cells. Indeed expression of both PPARα and PPARγ has been documented in

endothelial cells [Delerive et al. 1999, Marx et al. 1999, Inoue et al. 1998, Chinetti et al. 2000, 1998, Xin et al. 1999], SMCs [Staels et al. 1998, Iijima et al. 1998, Marx et al. 1998, Law et al. 2000] and monocytes/macrophages [Chinetti et al. 1998, Ricote et al. 1998, Tontonoz et al. 1998, Marx et al. 1998]. In contrast to PPARα which is already expressed by human monocytes, PPARγ is not detectable in human monocytes but is strongly induced upon differentiation of monocytes into macrophages [Chinetti et al. 1998].

LPL is also induced during the differentiation of monocytes [Chait et al. 1982]. Various lines of evidence have suggested that LPL secreted by macrophages and by macrophage-derived foam cells in the vessel wall [Yla-Herttuala et al. 1991, O'Brien et al. 1992] favors foam cell formation and atherosclerosis in vivo [Babaev et al. 1999, idem 2000, Semenkovich et al. 1998, Van Eck et al. 2000]. Macrophage LPL may promote atherosclerosis through a variety of mechanisms, including 1) monocyte recruitment and retention in the artery wall [Saxena et al. 1992, Mamputu et al. 1997, Obunike et al, 1997], 2) promotion of lipoprotein uptake by macrophages [Lindqvist et al. 1983, Aviram et al. 1987, Valdimir et al. 1999], and 3) increased retention of lipoproteins in the extracellular matrix through LPL-mediated bridging with proteoglycans [Saxena et al. 1992, Susanne et al. 2000].

LPL and PPARs share many features. Both factors play a key role in lipid metabolism and are expressed by macrophages in the arterial wall. Both also exhibit a parallel induction during the differentiation process of monocytes into

macrophages and have been shown to play a key role in arterial lipid metabolism and foam cell formation [Babaev et al. 1999, Tontonoz et al. 1998].

Our finding that PPAR ligands upregulate human macrophage LPL expression further supports the possibility that PPAR expressed in the arterial wall may regulate the production of macrophage LPL at vascular sites. Based on the key role of macrophage LPL in foam cell formation and on previous observations showing that PPARγ activators may favor influx of lipids in macrophages, one may postulate that induction of LPL may represent one mechanism involved in PPAR-induced lipid uptake in macrophages. Future experiments aimed at comparing cholesterol influx in macrophages that are LPL-/- vs LPL +/+ in response to PPAR ligands will be conducted to evaluate this possibility.

Evidence that PPAR ligands stimulate macrophage LPL secretion further supports the possibility that PPARs are important modulators of atherosclerosis development. Despite the accumulating evidence showing that PPARs affect vascular cell function, there is still a considerable debate regarding the role of these compounds in atherogenesis. Indeed, many studies have demonstrated that PPARs may exert both pro- and anti-atherogenic effects. When considering the role of PPARα agonists in atherogenesis, several studies have demonstrated the potential of these compounds to regulate the inflammatory processes in the arterial wall. Activation of PPARα has been found to potentiate TNF-α–induced human macrophage apoptosis [Chinetti et al. 1998] and to increase CLA-1/SR-BI expression [Chinetti et al. 1999]. Both effects may result in reduced foam cell

content and activity in atherosclerotic lesions. Activation of PPARα has also been documented to inhibit IL-6 secretion from mouse vessel wall ex vivo [Delerive et al. 1999], monocyte binding to human aortic endothelial cells and VCAM-1 expression on the endothelial cell surface [Jackson et al. 1999, Marx et al. 1999]. This latter effect has been suggested to result from the negative cross-talk between PPARα and the transcription factor, NF-κB [Marx et al. 1999]. Such inhibition is consistent with the recent findings of Delerive et al showing that fibrates inhibit the vascular inflammatory response via PPARα by interfering with NF-κB and AP-1 transactivation capacity [Delerive et al. 1999]. It has also been reported that PPARα ligands repress thrombin-induced expression of endothelin-1 (ET-1), a potent vasoconstrictor peptide and inducer of SMC proliferation [Delerive et al. 1999]. Activation of PPARα has also been shown to inhibit inflammatory responses in vascular SMC. Indeed evidence has been provided that PPARα ligands inhibit cytokine-induced IL-6 production and cycloxygenase-2 expression in these cells [Staels et al. 1998]. The anti-inflammatory effects of PPARα agonists on vascular SMC appear to be mirrored in vivo as suggested by the reduction of plasma levels of IL-6 following administration of fenofibrate to patients with hyperlipidemia [Satels et al. 1998). Further support for the anti-atherosclerotic effects of PPARα agonists comes from recent studies showing that fenofibrate decreases atherosclerotic plaque formation [Saitoh et al. 1995, Fruchart et al. 2001].

In contrast, several reports suggest some pro-inflammatory effects of PPARα. For example, it has been reported that the natural PPARα ligands LTB4

and 8(S)-HETE stimulate nitrite accumulation in monocytes/macrophages, suggesting that these agonists may exert some pro-inflammatory properties in the arterial wall [Colville-Nash et al. 1998]. Hill et al. [1999] found that in mice, fibrates markedly increase plasma TNF-α levels. Recently, Hans et al. [2000] demonstrated that PPARα plays a role in mediating the effects of oxidized phospholipids on endothelial cell synthesis of the monocyte chemoattractants, MCP-1 and IL-8. Finally, Sartippour et al. [2000] recently suggested that PPARα could mediate the stimulatory effect of high glucose on macrophage LPL gene expression.

Many studies have also examined the influence of PPARγ in atherosclerosis. Studies aimed at examining the role of PPARγ activators on macrophage function have shown that these agents promote macrophage apoptosis [Chinetti et al. 1998] and favor the differentiation of human monocytes and the uptake of oxLDL by macrophages through the induction of the oxLDL receptor CD36 [Tontonoz et al. 1999]. It has also been reported that constituents of oxLDL and products of 15-lipoxygenase activity promote foam cell formation and macrophage gene expression through PPARγ activation [Nagy et al. 1998]. Moreover, it has been documented that constituents of oxLDL and products of 15-lipoxygenase activity promote foam cell formation and macrophage gene expression through PPARγ activation [Nagy et al. 1998]. Another study from Huang et al also reported that IL-4 mediates CD36 expression via the induction of PPARγ [Huang et al. 1999]. Based on these results it has been proposed that oxLDL and PPARγ are involved in a positive feedback loop in which oxLDL

induces PPARγ expression leading to increased CD36 expression which increases oxLDL uptake, subsequently reinforcing the positive feedback cycle. If these effects are reproduced in vivo, then activation of PPARγ could contribute to the development of foam cells. An explosion of new information has led to the concept that PPARγ ligands affect both the influx and efflux of cholesterol and oxidized lipids in macrophages, stimulating cholesterol removal from macrophages through downregulation of SR-A scavenger receptor [Moore et al. 2001] and stimulation of the ABCA1 pathway [Chinetti et al. 2001]. Recent observations showing that treatment of low density lipoprotein receptor-deficient or apoE knockout mice with PPARγ ligands inhibits fatty streak lesion formation seem to support a net in vivo anti-atherogenic effect of PPARγ activation in foam cells [Li et al. 2000, Chen et al. 2001]. PPARγ has also been found to inhibit macrophage activation as demonstrated by the reduction of gelatinase B, inducible nitric oxide synthase activity/expression and TNF-α, IL-1β, and IL-6 production. [Ricote et al 1998, Tontonoz et al. 1998]. Some of these effects have been related to the inhibitory effect of PPARγ ligands on the activity of pro-inflammatory transcription factors including AP-1, STAT and NF-κB. PPARγ agonists also reduced the secretion and gelatinolytic activity of the metalloproteinase MMP-9 [Marx et al. 1999, 1998]. This anti-inflammatory effect of PPARγ could be beneficial in the treatment of atherosclerosis. PPARγ ligands also modulate endothelial cell function. For example, both inhibitory and stimulatory effects of these agents on plasminogen activator inhibitor-I release have been reported [Kato et al. 1999, Marx et al. 1999] and the ability of PPARγ

agonists to inhibit secretion of ET-1 has been reported [Satoh et al. 1999, Delerive et al. 1999]. These ligands also inhibit monocyte adhesion and the expression of the endothelial cell adhesion molecule, VCAM-1 [Jackson et al. 1999]. Furthermore, they could reduce monocyte recruitment into the artery wall by inhibiting endothelial cell production of MCP-1 [Murao et al. 1999]. It has also been reported that the PPARγ activators pioglitazone and troglitazone inhibit in experimental models neo-intimal thickening, hypertrophy and hyperplasia [Dubey et al. 1993, Law et al. 1996, Igarashi et al. 1997, Shinohara et al. 1998]. While the ability of PPARγ agonists to inhibit SMC proliferation has been well documented [Dubey et al. 1993, Law et al. 1996, Morikang et al. 1997], these compounds also reduce SMC migration [Marx et al. 1998] and calcium entry in these cells [Zhang et al 1994, Song et al. 1997, Asano et al. 1999]. Recently, it has been shown that PPARγ ligands reduce carotid artery intima and media thickness in patients with type 2 diabetes [Minamikawa et al. 1998] and inhibit development of atherosclerosis in LDL receptor-deficient mice [Li et al. 2000]. Interestingly, Sartippour et al. [2000] reported a reduction of PPARγ mRNA expression in MDM isolated from patients with type 2 diabetes. A similar alteration was observed in human macrophages exposed in vitro to high glucose concentrations [Sartippour et al. 2000].

Overall, these data indicate that PPAR agonists may directly modulate vessel wall function and that these agents are likely important modulators of atherosclerosis development. Whether our results showing the ability of PPAR activators to stimulate macrophage LPL expression indicates a pro- or an anti-

atherogenic effect of PPARα agonists is presently unknown. While such an effect may be considered as "protective" in the early stage of atherogenesis, it may also contribute later to increased foam cell formation and activity in atherosclerotic lesions.

IV. PERSPECTIVES AND CONCLUSION

IV.1. Perspectives

Although we have demonstrated that PPARs increase macrophage LPL expression both at the gene and protein levels, the functional relevance of the upregulation of macrophage LPL by PPARs remains unclear. To address this question, we propose to conduct future studies aimed at examining macrophage foam cell formation in LPL-/- vs LPL +/+ mice in response to PPAR agonists. These studies will also allow to identify the role of LPL in PPAR-induced foam cell formation.

Experiments aimed at determining the molecular mechanisms involved in the induction of macrophage LPL gene expression by PPARs will also be conducted. In particular the possibility that these agents may regulate this gene by interacting with other nuclear factors, such as stimulating protein-1, will be evaluated.

Finally, we propose to further investigate the mechanisms involved in the differential regulation of macrophage PPAR expression in human type 2 diabetes [Sartippour et al. 2000]. Decreased macrophage PPARγ expression in diabetes may theoretically involve TNF-α. Indeed it has been shown that high glucose stimulates TNF-α secretion [Morohoshi et al. 1995] and that this cytokine, in turn, exerts a suppressive effect on PPARγ expression [Xing et al. 1997, Zhang et al. 1996]. Because advanced glycation end products accumulate in the blood and tissues of patients with diabetes and that these products increase the production of human monocyte and macrophage TNF-α [Morohoshi et al. 1996, Sartippour et al. 1998] and induce PPAR expression in mesangial cells [Iwashima et al.

1999], it will also be of major interest to characterize in the future the modulatory effect of these factors on macrophage PPAR expression.

IV.2. Conclusion

The present study provides the first evidence of a direct regulatory effect of PPAR agonists on human macrophage LPL gene expression and secretion. Our data showing that PPARs control macrophage LPL expression further stress the crucial role of these factors in the regulation of arterial lipid metabolism. The finding that PPARs agonists increase macrophage LPL production suggests a new mechanism by which these agents may favor foam cell formation and influence atherosclerosis. A detailed understanding of the role of PPARs in the regulation of vascular biology and the development of novel PPAR ligands that dissociate pro- and anti-atherogenic properties will offer new strategies in the treatment of atherosclerosis.

V. REFERENCES

References

Abumrad NA, El-Maghrabi MR, Amri E-Z, Lopez E, Grimaldi PA. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. *J Biol Chem* 1993, 268: 17665–17668.

Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. *N Engl J Med* 1995, 332:488-493.

Aoyama T, Peters JM, Iritani N, Nakajima T, Furihata K, Hashimoto T, Gonzalez FJ. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *J Biol Chem* 1998, 273 :5678-5684.

Asano M, Nakajima T, Iwasawa K, Morita T, Nakamura F, Imuta H, Chisaki K, Yamada N, Omata M, Okuda Y. Troglitazone and pioglitazone attenuate agonist-dependent Ca2+ mobilization and cell proliferation in vascular smooth muscle cells. *Br J Pharmacol* 1999, 128:673-683.

Auboeuf D, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M, Vidal H. Tissue distribution and quantification of the expression of

mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes*. 1997, 46:1319-1327.

Auerbach BJ, Bisgaier CL, Wolle J, Saxena U. Oxidation of low density lipoproteins greatly enhances their association with lipoprotein lipase anchored to endothelial cell matrix. *J Biol Chem* 1996, 271:1329-1335.

Aviram M, Fuhrman B, Boulos M, Ginsburg H, Brook JG. Effect of plasma lipoproteins on cholesterol accumulation in macrophages: comparison of lipoproteins from normal and homozygous familial hypercholesterolemic subjects. *Atherosclerosis* 1987, 65:207-214.

Aviram M. Macrophage foam cell formation during early atherogenesis is determined by the balance between pro-oxidants and anti-oxidants in arterial cells and blood lipoproteins. *Antioxid Redox Signal* 1999, 1:585-594.

Babaev VR, Fazio S, Gleaves LA, Carter KJ, Semenkovich CF, Linton MF. Macrophage lipoprotein lipase promotes foam cell formation and atherosclerosis in vivo. *J Clin Invest* 1999, 103:1697-1705.

Babaev VR, Patel MB, Semenkovich CF, Fazio S, Linton MF. Macrophage

lipoprotein lipase promotes foam cell formation and atherosclerosis in low density lipoprotein receptor-deficient mice. *J Biol Chem* 2000, 275:26293-26299.

Bae J, Bassenge E, Kim K, Kim Y, Kim K, Lee H, Moon K, Lee M, Park K, Schwemmer M. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis* 2001, 155:517-523.

Barath P, Fishbein MC, Cao J, Berenson J, Helfant RH, Forrester JS. Detection and localization of tumor necrosis factor in human atheroma. *Am J Cardiol* 1990, 65:297-302.

Barcellini-Couget S, Doglio A, Dani C.Regulation of c-fos proto-oncogene expression by growth hormone: protein synthesis is not required for down-regulation. *Endocrinology* 1993, 132:1875-1877.

Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 1990, 87:1620-1624.

Behr SR, Kraemer FB. Insulin deficiency decreases lipoprotein lipase secretion by murine macrophages. *Diabetes* 1988, 37:1076-1081.

Behr SR, Kraemer FB. Regulation of the secretion of lipoprotein lipase by mouse macrophages. *Biochim Biophys Acta* 1986, 889:346-354.

Beisiegel U, Weber W, Bengtsson-Olivecrona G. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc Natl Acad Sci U S A* 1991, 88:8342-8346.

Benlian P, De Gennes JL, Foubert L, Zhang H, Gagne SE, Hayden M. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. *N Engl J Med* 1996, 335:848-854.

Ben-Zeev O, Doolittle MH, Davis RC, Elovson J, Schotz MC. Maturation of lipoprotein lipase. Expression of full catalytic activity requires glucose trimming but not translocation to the cis-Golgi compartment. *J Biol Chem* 1992, 267:6219-6227.

Berger J, Balley P, Biswas C, Cullinan CA, Doebber TW, Hayes NS, Saperstein R, Smith RG, Leibowita MD. Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-γ: binding and activation correlate with antidiabetic actions in db/db mice. *Endocrinology*. 1996, 137:4189-4195.

Beutler BA, Cerami A. Recombinant interleukin 1 suppresses lipoprotein lipase activity in 3T3-L1 cells. *J Immunol* 1985, 135:3969-3971.

Beutler BA, Milsark IW, Cerami A. Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. *J Immunol* 1985, 135:3972-3977.

Bevilacqua MP, Pober JS, Majeau GR, Fiers W, Cotran RS, Gimbrone MA Jr. Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. *Proc Natl Acad Sci U S A* 1986, 83:4533-4537.

Bishop-Bailey D, Hla T. Endothelial cell apoptosis induced by the peroxisome proliferator-activated receptor (PPAR) ligand 15-deoxy 12,14 prostaglandin J2. *J Biol Chem* 1999, 274:17042-17048.

Boisvert WA, Santiago R, Curtiss LK, Terkeltaub RA. A leukocyte homologue of the IL-8 receptor CXCR2 mediates the accumulation of macrophage in atherosclerotic lesions of LDL receptor-deficient mice. *J Clin Invest* 1998, 101:353-363.

Boring L, Gosling J, Chensue SW, Kunkel SL, Farese RV, Broxmeyer HE, Charo IF. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. *J Clin Invest* 1997, 100:2552-2561.

Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 1996, 137:354-366.

Braissant O, Wahli W. Differential expression of peroxisome proliferator-activated receptor-alpha, -beta, and -gamma during rat embryonic development. *Endocrinology* 1998, 139:2748-2754.

Brandt J, Djouadi F, Kelly D. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. *J Biol Chem* 1998, 273:23786–23792.

Brattstrom L, Wilcken DE. Homocysteine and cardiovascular disease: cause or effect? *Am J Clin Nutr* 2000, 72:315-323.

Breckenridge WC, Little JA, Steiner G, Chow A, Poapst M. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. *N Engl J Med* 1978, 298:1265-1273.

Brown G, Albers JJ, Fisher LD, Schaefer SM, Lin JT, Kaplan C, Zhao XQ, Bisson BD, Fitzpatrick VF, Dodge HT. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. *N Engl J Med* 1990, 323:1289-1298.

Brown MS, Goldstein JI. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem* 1983, 52:223-2261.

Casrroll R, Ben-Zeev O, Doolittle MH, Severson DL. Activation of lipoprotein lipase in cardiac myocytes by glycosylation requires trimming of glucose residues in the endoplasmic reticulum. *Biochem J* 1992, 285:693-696.

Castelli WP, Levy D, Wilson PW, Anderson K, Sanders M. Sudden death: the view from Framingham. In: the prevention of sudden cardiac death. Kostis JB, Sanders M, eds. Wiley Liss, New York 1990:1-12.

Castrillo A, Diaz-Guerra MJ, Hortelano S, Martin-Sanz P, Bosca L. Inhibition of IkappaB kinase phosphorylation by 15-deoxy-Delta (12,14)-prostaglandin J(2) in activated murine macrophages. *Mol Cell Biol* 2000, 20:1692–1698.

Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992, 340:1111-1115.

Cernacek P, Stewart DJ. Immunoreactive endothelin in human plasma: marked elevations in patients in cardiogenic shock. *Biochem Biophys Res Commun* 1989, 161:562-567.

Chait A, Iverius PH, Brunzell JD. Lipoprotein lipase secretion by human monocyte-derived macrophages. *J Clin Invest* 1982, 69:490-493.

Chappell DA, Fry GL, Waknitz MA, Muhonen LE, Pladet MW, Iverius PH, Strickland DK. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor in vitro. A process facilitated by cell-surface proteoglycans. *J Biol Chem* 1993, 268:14168-14175.

Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* 1994, 135:798-800.

Chen NG, Sarabia SF, Malloy PJ, Zhao XY, Feldman D, Reaven GM. PPAR gamma agonists enhance human vascular endothelial adhesiveness by increasing ICAM-1 expression. *Biochem Biophys Res Commun* 1999, 263:718-722.

Cheng Q, Blackett P, Jackson KW, McConathy WJ, Wang CS. C-terminal domain of apolipoprotein CII as both activator and competitive inhibitor of lipoprotein lipase. *Biochem J* 1990, 269:403-407.

Chen Z, Ishibashi S, Perrey S, Osuga Ji, Gotoda T, Kitamine T, Tamura Y, Okazaki H, Yahagi N, Iizuka Y, Shionoiri F, Ohashi K, Harada K, Shimano H, Nagai R, Yamada N. Troglitazone inhibits atherosclerosis in apolipoprotein E-knockout mice: pleiotropic effects on CD36 expression and HDL. Arterioscler Thromb Vasc Biol 2001, 21:372-7.

Chinetti G, Gbaguidi GF, Griglio S, Mallat Z, Antonucci M, Poulain P, Chapman J, Fruchart JC, Tedgui A, Najib-Fruchart J, Staels B. CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. *Circulation* 2000, 101:2411-2417.

Chinetti G, Griglio S, Antonucci M, Pineda Torra I, Delerive P, Majd Z et al. Activation of peroxisome proliferator-activated receptors α and γ induces apotosis of human monocyte-derived macrophages. *J Biol Chem* 1998, 273: 25573-25580.

Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, Teissier E, Minnich A, Jaye M, Duverger N, Brewer HB, Fruchart JC, Clavey V, Staels B. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat Med* 2001, 7:53-8.

Chobanian AV, Dzau VJ. Renin angiotensin system and atherosclerotic vascular disease. *In:Fuster V, Toss R, Topol EJ, eds. Atherosclerosis and coronary artery disease. Vol. 1. Philadelphia: Lippincott-Raven* 1996, 273-342.

Choen R. The role of nitric oxide and other endothelium-derived vasoactive substances in vascular disease. *Prog Cardiovasc Dis* 1995, 38:105-128.

Cisar LA, Hoogewerf AJ, Cupp M, Rapport CA, Bensadoun A. Secretion and degradation of lipoprotein lipase in cultured adipocytes. Binding of lipoprotein lipase to membrane heparan sulfate proteoglycans is necessary for degradation. *J Biol Chem* 1989, 264:1767-1774.

Colville-Nash PR, Qureshi SS, Willis D, Willoughby DA. Inhibition of inducible nitric oxide synthase by peroxisome proliferator-activated receptor agonists: correlation with induction of heme oxygenase 1. *J Immunol* 1998, 161:978-984.

Corey JE, Zilversmit DB. Effect of cholesterol feeding on arterial lipolytic activity in the rabbit. *Atherosclerosis* 1977, 27:201-212.

Cornelius P, Enerback S, Bjursell G, Olivecrona T, Pekala PH. Regulation of lipoprotein lipase mRNA content in 3T3-L1 cells by tumour necrosis factor. *Biochem J* 1988, 249:765-769.

Couturier C, Brouillet A, Couriaud C, Koumanov K, GB, Andriani M. Interleukin 1 beta induces type II-secreted phospholipase A2 gene in vascular smooth muscle cells by a nuclear factor kappaB and peroxisome proliferator-activated receptor-mediated process. *J Biol Chem* 1999, 274:23085-23093.

Currie RA, Eckel RH. Characterization of a high affinity octamer transcription factor binding site in the human lipoprotein lipase promoter. *Arch Biochem Biophys* 1992, 298:630-639.

Dalton ML, Gadson PF Jr, Wrenn RW, Rosenquist TH. Homocysteine signal cascade: production of phospholipids, activation of protein kinase C, and the induction of c-fos and c-myb in smooth muscle cells. *FASEB J* 1997, 11:703-711.

Danesh J, Clllins R, Peto R. Chronic infections and coronary heart disease: is there a link? *Lancet* 1997, 350:430-436.

Davies MJ. A macro and micro view of coronary vascular insult in ischemic heart disease. *Circulation* 1990, 82:1138-1146.

de Boer OJ, Hirsch F, van der Wal AC, van der Loos CM, Das PK, Becher AE. Costimulatory molecules in human atherosclerotic plaques: an indication of antigen specific T lymphocyte activation. *Atherosclerosis* 1997, 133:227-234.

Deeb SS, Peng RL. Structure of the human lipoprotein lipase gene. *Biochemistry* 1989, 28:4131-4135.

Delerive P, De Bosscher K, Besnard S, Vanden berghe W, Prters JM, Gonzalez FJ et al. PPARα negatively regulates the vascular wall inflammatory gene response by negative cross-talk with transcription factors NF-κB and AP-1. *J Biol Chem* 1999, 274:32048-32054.

Delerive P, Martin-Nizard F, Chinetti G, Trottein F, Fruchart JC, Najib J, Duriez P, Staels B. Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. *Circ Res* 1999, 85:394-402.

Deng X, King M, Guidoin R. Localization of atherosclerosis in arterial junctions. Modeling the release rate of low density lipoprotein and its breakdown products accumulated in blood vessel walls. *ASAIO J* 1993, 39:489-95.

de Sanctis JB, Blanca I, Radzioch D, Bianco NE. Lipoprotein lipase expression in natural killer cells and its role in their cytotoxic activity. *Immunology* 1994, 83:232-239.

Despres JP, Pascot A, Lemieux I. Risk factors associated with obesity: a metabolic perspective. *Ann Endocrinol* 2000, 6:31-38.

Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The PPAR α -leukotriene B4 pathway to inflammation control. *Nature* 1996, 384:39-43.

Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens* 2000, 18:655-673.

Djurovic S, Berg K. Epidemiology of Lp(a) lipoprotein: its role in atherosclerotic/thrombotic disease. *Clin Genet* 1997, 52:281-292.

Ding ST, McNeel RL, Mersmann HJ. Expression of porcine adipocyte transcripts: tissue distribution and differentiation in vitro and in vivo. *Comp Biochem Physiol B Biochem Mol Biol* 1999, 123:307-318.

Dobrian AD, Lazar V, Sinescu C, Mincu D, Simionescu M. Diabetic state induces lipid loading and altered expression and secretion of lipoprotein lipase in human monocyte-derived macrophages. *Atherosclerosis* 2000,153:191-201.

Dreyer C, Keller H, Mahfoudi A, Laudet V, krey G, Wahli W. Positive regulation of the peroxisomal β-oxidation pathway by fatty acids through activation of the peroxisome proliferator-activited receptor (PPAR). *Biol Cell* 1993, 77:67-76.

Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 1992, 4:281-299.

Dubey RK, Zhang HY, Reddy SR, Boegehold MA, Kotchen TA. Pioglitazone attenuates hypertension and inhibits growth of renal arteriolar smooth muscle in rats. *Am J Physiol* 1993, 265:726-732.

Durand P, Lussier-Cacan S, Blache D. Acute methionine load-induced hyperhomocysteinemia enhances platelet aggregation, thromboxane biosynthesis, and macrophage-derived tissue factor activity in rats. *FASEB J* 1997, 11:1157-1168.

Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med.*1989, 16:1060-1068.

Edwards K, Chan RYS, Sawyer WH. Interactions between fatty acids and lipoprotein lipase: specific binding and complex formation. *Biochemistry* 1994, 33:13304-13311.

Eisenberg s, Sehayek E, Olivecrona T, Vlodavsky I. Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. *J Clin Invest* 1992, 90:2013-2021.

Elkind MS, Lin IF, Grayston JT, Sacco RL. Chlamydia pneumoniae and the risk of first ischemic stroke : The Northern Manhattan Stroke Study. *Stroke* 2000, 31:1521-1525.

Enerbäck S, Gimble JM. Lipoprotein lipase gene expression: physiological regulators at the transcriptional and posttranscriptional level. *Biochim Biophys Acta* 1993, 1169:107-125.

Enerbäck S, Ohlsson BG, Samuelsson L, Bjursell G. Characterization of the human lipoprotein lipase (LPL) promoter: evidence of two cis-regulatory regions, LP-alpha and LP-beta, of importance for the differentiation-linked induction of the LPL gene during adipogenesis. *Mol Cell Biol* 1992, 12:4622-4633.

Enerback S, Semb H, Bengtsson-Olivecrona G, Carlsson P, Hermansson ML, Olivecrona T, Bjursell G. Molecular cloning and sequence analysis of cDNA encoding lipoprotein lipase of guinea pig. *Gene* 1987, 58:1-12.

Ericsson C, Hamsten A, Nilsson J, Grip L, Svane B, de Faire U. Angiographic

assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. *Lancet* 1996, 347:849-853.

Fajas L, Fruchar JC, Auwerx J. Transcriptional control of adipogenesis. *Curr Opin Cell Biol* 1998, 10:165-173.

Falkenberg M, Giglio D, Bjornheden T, Nygren H, Risberg B. Urokinase plasminogen activator colocalizes with $CD25^+$ cells in atherosclerotic vessels. *J Vasc Res* 1998, 35:318-324.

Fallavallita JA, Brody AS, Bunnell IL, Kumar K, Canty Jr. Fast computed tomography detection of coronary calcification in the diagnosis of coronary artery disease. Comparison with angiography in patients < 50 years old. *Circulation* 1994, 89:285-290.

Fan J, Shimoyamada H, Sun H, Marcovina S, Honda K, Watanabe T. Transgenic rabbits expressing human apolipoprotein(a) develop more extensive atherosclerotic lesions in response to a cholesterol-rich diet. *Arterioscler Thromb Vasc Biol* 2001, 21:88-94.

Farese RV, Yost TJ, Eckel RH. Tissue-specific regulation of lipoprotein lipase activity by insulin/glucose in normal-weight humans. *Metabolism* 1991, 40:214-216.

Farsak B, Yildirir A, Akyon Y, Pinar A, Oc M, Boke E, Kes S, Tokgozoglu L. Detection of chlamydia pneumoniae and helicobacter pylori DNA in human atherosclerotic plaques by PCR. *J Clin Microbiol* 2000, 38:4408-4411.

Fish RD, Nabel EG, Selwyn AP, Ludmer PL, Mudge GH, Kirshenbaum JM, Schoen FJ, Alexander RW, Ganz P. Responses of coronary arteries of cardiac transplant patients to acetylcholine. *J Clin Invest* 1988, 81:21-31.

Flier JS, Underhill LS. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 1989,1060-1068.

Frick MH, Syvanne M, Nieminen MS, Kauma H, Majahalme S, Virtanen V, Kesaniemi YA, Pasternak A, Taskinen MR. Prevention of the angiographic progression of coronary and vein-graft atherosclerosis by gemfibrozil after coronary bypass surgery in men with low levels of HDL cholesterol. *Circulation* 1997, 96:2137-2143.

Friedman G, Chajek-Shaul T, Olivecrona T, Stein O, Stein Y. Fate of milk ^{125}I-labelled lipoprotein lipase in cells in culture. Comparison of lipoprotein lipase- and non-lipoprotein lipase-synthesizing cells. *Biochim Biophys Acta* 1982, 711:114-

122.

Fruchart JC, Duriez P, Staels B. Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. *Curr Opin Lipidol* 1999,10:245-257.

Fruchart JC, Staels B, Duriez P. The Role of Fibric Acids in Atherosclerosis. *Curr Atheroscler Rep* 2001, 3:83-92.

Gagne SE, Larson MG, Pimstone SN, Schaefer EJ, Kastelein JJ, Wilson PW, Ordovas JM, Hayden MR. A common truncation variant of lipoprotein lipase (Ser447X) confers protection against coronary heart disease: the Framingham Offspring Study. *Clin Genet* 1999, 55:450-454.

Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 1994, 94:2493-2503.

Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc Natl Acad Sci U S A* 1985, 82:8667-8671.

Garcia MJ, McNamara PM, Gordon T, Kannel WB. Morbidity and mortality in

diabetics in the Framingham population. Sixteen year follow-up study. *Diabetes* 1974, 23:105-111.

Gervois P, Chopin-Delannoy S, Fadel A, Dubois G, Kosykh V, Fruchart JC, Najib J, Laudet V, Staels B. Fibrates increase human REV-ERB alpha expression in liver via a novel peroxisome proliferator-activated receptor response element. *Mol Endocrinol* 1999, 13:400-409.

Giachelli CM, Lombardi D, Johnson RJ, Murry CE, Almeida M. Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli in vivo. *Am J Pathol* 1998, 152:353-358.

Gibbons GH, Pratt RE, Dzau VJ. Vascular smooth muscle cell hypertrophy vs. hyperplasia. Autocrine transforming growth factor-beta 1 expression determines growth response to angiotensin II. *J Clin Invest* 1992, 90:456-461.

Goldberg, I.J. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res* 1996, 37:693-707.

Goldman R. Control of lipoprotein lipase secretion by macrophages: effect of macrophage differentiation agents. *J Leukoc Biol* 1990, 47:79-86.

Goldman R, Sopher O. Control of lipoprotein lipase secretion in mouse macrophages. *Biochim Biophys Acta* 1989, 1001:120-126.

Gotlieb AI, Langille BL. The role of rheology in atherosclerotic coronary artery disease. In*: Fuster V, Ross R, Topol EJ, eds. Atherosclerosis and coronary disease. Vol. 1. Philadelphia: Lippincott-Raven* 1996,595-606.

Gouni I, Oka K, Etienne J, Chan L. Endotoxin-induced hypertriglyceridemia is mediated by suppression of lipoprotein lipase at a post-transcriptional level. *J Lipid Res* 1993, 34:139-146.

Granneman J, Skoff R, Yang X. Member of peroxisome proliferator-activated receptor family of transcription factors is differentially expressed by oligodendrocytes. *J Neurosci Res* 1998, 51:563-573.

Gray JM, Wade GN. Cytoplasmic estrogen, but not progestin, binding sites in male rat adipose tissues. *Am J Physiol* 1980, 239:237-241.

Greenberg AS, Nordan RP, McIntosh J, Calvo JC, Scow RO, Jablons D. Interleukin 6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3T3-L1 adipocytes: a possible role for interleukin 6 in cancer cachexia. *Cancer Res* 1992, 52: 4113-4116.

Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, Hsieh L, Greene G, Nimer SD. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. *Gene Expression* 1995, 4:281-299.

Gregoire F, De Broux N, Hauser N, Heremans H, Van Damme J, Remacle C. Interferon-gamma and interleukin-1 beta inhibit adipoconversion in cultured rodent. *J Cell Physiol* 1992, 151:300-309.

Griendling KK, Alexander RW. Oxidative stress and cardiovascular disease. *Circulation* 1997, 96:3264-3265.

Grunfeld C, Adi S, Soued M, Moser A, Fiers W, Feingold KR. Search for mediators of the lipogenic effects of tumor necrosis factor: potential role for interleukin 6. *Cancer Res* 1990, 50:4233-4238.

Gurfinkel E, Bozovich G, Beck E, Testa E, Livellara B, Mautner B. Treatment with the antibiotic roxithromycin in patients with acute non-Q-wave coronary syndromes. The final report of the ROXIS Study. *Eur Heart J* 1999, 20:121-127.

Guyton AC, Hall JE. Textbook of Medical Physiology. W.B. Saunders Company. *Toronto. Lipid Metabolism* 1996, 865-875.

Han KH, Chang MK, Boullier A, Green SR, Li A, Glass CK, Quehenberger O. Oxidized LDL reduces monocyte CCR2 expression through pathways involving peroxisome proliferator-activated receptor gamma. *J Clin Invest* 2000, 106:793-802.

Han J, Hajjar DP, Febbraio M, Nicholson AC. Native and modified low density lipoproteins increase the functional expression of the macrophage class B scavenger receptor, CD36. *J Biol Chem* 1997, 272:21654-21659.

Hansson GK. Immunological control mechanisms in plaque formation. *Basic Res Cardiol* 1994, 89:41-46.

Harrison DG, Armstrong ML, Freiman PC, Herstad DD. Restoration of endothelium-dependent relaxation by dietary treatment of atherosclerosis. *J Clin Invest* 1987, 80:1808-1811.

Harrison DG. Endothelial function and oxidant stress. *Clin Cardiol* 1997, 20:11-17.

Hattori Y, Hattori S, Kasai K. Troglitazone upregulates nitric oxide synthesis in vascular smooth muscle cells. *Hypertension* 1999, 33:943-948.

Henderson HE, Kastelein JJ, Zwinderman AH, Gagne E, Jukema JW, Reymer PW, Groenemeyer BE, Lie KI, Bruschke AV, Hayden MR, Jansen H. Lipoprotein lipase activity is decreased in a large cohort of patients with coronary artery disease and is associated with changes in lipids and lipoproteins. *J Lipid Res* 1999, 40:735-743.

Hendrix MG, Salimans MM, van Boven CP, Bruggeman CA. High prevalence of latently present cytomegalovirus in arterial walls of patients suffering from grade III atherosclerosis. *Am J Pathol* 1990, 136:23-28.

Henrion D, Dowell FJ, Levy BI, Michel JB. In vitro alteration of aortic vascular reactivity in hypertension induced by chronic *N* G -nitro-L-arginine methyl ester. *Hypertensio*n. 1996;28:361-366.

Hikita M, Bujo H, Yamazaki K, Taira K, Takahashi K, Kobayashi J, Saito Y. Differential expression of lipoprotein lipase gene in tissues of the rat model with visceral obesity and postprandial hyperlipidemia. *Biochem Biophys Res Commun* 2000, 277:423-429.

Hill MR, Clarke S, Rodgers K, Thornhill B, Peters JM, Gonzalez FJ, Gimble JM. Effect of peroxisome proliferator-activated receptor alpha activators on tumor necrosis factor expression in mice during endotoxemia. *Infect Immun* 1999, 67:3488-3493.

Hilton DJ, Gough NM. Leukemia inhibitory factor: a biological perspective. *J Cell Biochem* 1991, 46:21-26.

Hua XX, Enerback S, Hudson J, Youkhana K, Gimble JM. Cloning and characterization of the promoter of the murine lipoprotein lipase-encoding gene: structural and functional analysis. *Gene* 1991, 107:247-258.

Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C, Witztum JL, Funk CD, Conrad D, Glass CK. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. *Nature* 1999, 400:378-382.

Hulten LM, Lindmark H, Diczfalusy U, Bjorkhem I, Ottosson M, Liu Y, Bondjers G, Wiklund O. Oxysterols present in atherosclerotic tissue decrease the expression of lipoprotein lipase messenger RNA in human monocyte-derived macrophages. *J Clin Invest* 1996, 97:461-468.

Ijpenberg a, Jeannin E, Wahli W, Desvergne B. Polarity and specific sequence requirements of PPAR-RXR heterodimer binding to DNA: a functional analysis of the malic enzyme gene PPRE. *J Biol Chem* 1997, 272:20108-20117.

Inoue I, Shino K, Noji S, Awata T, Katayama S. Expression of peroxisome proliferator-activated receptor α (PPARα) in primary cultures of human vascular endothelial cells. *Biochem Biophys Res Commun* 1998, 246:370-374.

Ishibashi S, Mori N, Murase T, Shimano H, Gotohda T, Kawakami M, Akanuma Y, Takaku F, Yamada N. Enhanced lipoprotein lipase secretion from human monocyte-derived macrophages caused by hypertriglyceridemic very low density lipoproteins. *Arteriosclerosis* 1989, 9:650-655.

Ishibashi S, Yamada N, Shimano H, Mori N, Mokuno H, Gotohda T, Kawakami M, Murase T, Takaku F. Apolipoprotein E and lipoprotein lipase secreted from human monocyte-derived macrophages modulate very low density lipoprotein uptake. *J Biol Chem* 1990, 265:3040-3047.

Issemann I, Green S. Activation of member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990, 347:645-650.

Iverius PH, Brunzell JD. Human adipose tissue lipoprotein lipase: changes with feeding and relation to postheparin plasma enzyme. *Am J Physiol* 1985, 249:107-114.

Iwashima Y, Eto M, Horiuchi S, Sano H. Advanced glycation end product-induced peroxisome proliferator-activated receptor gamma gene expression in the cultured mesangial cells. *Biochem Biophys Res Commun* 1999, 264:441-448.

Jackson CL, Bruns GA, Breslow JL. Isolation and sequence of a human apolipoprotein CII cDNA clone and its use to isolate and map to human chromosome 19 the gene for apolipoprotein CII. *Proc Natl Acad Sci USA* 1984, 81:2945-2949.

Jackson LA, Campbell LA, Schmidt RA, Kuo CC, Cappuccio AL, Lee MJ, Grayston JT. Specificity of detection of Chlamydia pneumoniae in cardiovascular atheroma: evaluation of the innocent bystander hypothesis. *Am J Pathol* 1997, 150:1785-1790.

Jackson SM, Parhami F, Xi XP, Berliner JA, Hsueh WA, Law RE, Demer LL. Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction. *Arterioscler Thromb Vasc Biol* 1999, 19:2094-2104.

Jang IK, Lassila R, Fuster V. Atherogenesis and inflammation. *Eur Heart J* 1993, 14:2-6.

Janowitz WR. CT imaging of coronary artery calcium as an indicator of atherosclerotic disease: an overview. *J Thorac Imaging* 2001, 16:2-7.

Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998, 391:82–86.

Jonasson L, Hansson GK, Bondjers G, Noe L, Etienne J. Interferon-gamma inhibits lipoprotein lipase in human monocyte-derived macrophages. *Biochim Biophys Acta* 1990, 1053:43-48.

Jurgens G, Chen Q, Esterbauer H, Mair S, Ledinski G, Dinges HP. Immunostaining of human autopsy aortas with antibodies to modified apolipoprotein B and apoprotein (a). *Arterioscler Throm*b 1993,13:1689-1699.

Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK, Rosenfeld MG. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* 1996, 85:403-414.

Karpe F, Olivcrona G, Samra JS, Summers LKM, Humphreys SM, Frayn KN. Lipoprotein lipase transport in plasma: role of muscle and adipose tissues in regulation of plasma lipoprotein lipase concentrations. *J Lipid Res* 1998, 33:974-984.

Kaplan M, Aviram M. Oxidized LDL binding to a macrophage-secreted extracellular matrix. *Biochem Biophys Res Commun* 1997, 237:271-276.

Kawakami M, Kondo Y, Hashiguchi M, Ogawa H, Hiragun A, Aotsuka S, Shibata S, Oda T, Murase T, Takaku F. Suppression of lipoprotein lipase in 3T3-L1 cells by a mediator produced by SEKI melanoma, a cachexia-inducing human melanoma cell line. *J Biochem* 1991, 109:78-82.

Kawashima I, Ohsumi J, Mita-Honjo K, Shimoda-Takano K, Ishikawa H, Sakakibara S, Miyadai K, Takiguchi Y. Molecular cloning of cDNA encoding adipogenesis inhibitory factor and identity with interleukin-11. *FEBS Lett* 1991, 283:199-202.

Keller H, Dreyer C, Medin J, Mahfoudi A, Ozato K, Wahli W. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc Natl Acad Sci USA* 1993, 90:2160-2164.

Khoo JC, Miller E, McLoughlin P, Steinberg D. Enhanced macrophage uptake of low density lipoprotein after self-aggregation. *Arteriosclerosis* 1998, 8:348-858.

Khoo JC, Miller E, Pio F, Steinverg D, Witztum JL. Monoclonal antibodies against LDL further enhance macrophage uptake of LDL aggregates. *Arterioscler Thromb* 1992, 12:1258-1266.

Kirchgessner TG, Chaut JC, Heinamann C, Etienne J, Guilhot S, Svenson K, Ameis D, Pilon C, d' Auriol L, Andalibi A, Schotz MC, Galibert F, Lusis AJ. Organization of the human lipoprotein lipase gene and evolution of the lipase gene family. *Proc Natl Acad Sci USA* 1989, 86:9647-9651.

Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdore DJ, Jmesono K, Evans RM. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci USA* 1994, 91:7355-7359.

Kothari K, Mathur A, Yadav S. Estimation of fasting and post oral glucose serum insulin levels in hypertensive and obese subjects. *J Assoc Physicians India* 1998, 46:514-517.

Krey G, Mahfoudi A, Wahli W. Functional interactions of peroxisome proliferator-activated receptor, retinoid-X receptor, and Sp1 in the transcriptional regulation of the acyl-coenzyme-A oxidase promoter. *Mol Endocrinol* 1995, 9:219-231.

Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S,

Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S, Kadowaki T et al. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol Cell* 1999, 4:597-609.

Kukreja RC, Hess ML. Free radicals, cardiovascular dysfunction and protection strategies. *Austin: RG Landes Company* 1994.

Kukreja RC, Hess ML. The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. *Cardiovasc Res* 1992, 26:641-655.

Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol* 1982, 157:105-132.

Ladu MJ, Kapsas H, Palmer WK. Regulation of lipoprotein lipase in adipose and muscle tissues during fasting. *Am J Physiol* 1991, 260:953-959.

Lai E, Prezioso VR, Tao WF, Chen WS, Darnell JE. Hepatocyte nuclear factor 3 alpha belongs to a gene family in mammals that is homologous to the Drosophila homeotic gene fork head. *Genes Dev* 1991, 5:416-427.

Lassila R. Inflammatory alterations in the myocardial microcirculation. *J Mol Cell Cardiol* 1993, 30:2555-2559.

Lawn R, Wade DP, Hammer RE, Chiesa G, Verstuyft JG, Rubin EM. Atherosclerosis in transgenic mice expressing human apolipoprotein (a). *Nature* 1992, 360:670-672.

Law RE, Goetze S, Xi XP, Jackson S, Kawano Y, Demer L, Fishbein MC, Meehan WP, Hsueh WA. Expression and function of PPAR gamma in rat and human vascular smooth muscle cells. *Circulation* 2000, 101:1311-1318.

Law RE, Meehan WP, Xi XP, Graf K, Wuthrich DA, Coats W, Faxon D, Hsueh WA Troglitazone inhibits vascular smooth muscle cell growth and intimal hyperplasia. *J Clin Invest* 1996, 98:1897-905.

Lee WJ, Sheu WH, Jeng CY, Young MS, Chen YT. Associations between lipoprotein lipase gene polymorphisms and insulin resistance in coronary heart disease. *Chung Hua I Hsueh Tsa Chih* 2000 , 63:563-572.

Lefebvre AM, Peinado-Onsurbe J, Leitersdorf I, Briggs MR, Paterniti JR, Fruchart JC, Fievet C, Auwerx J, Staels B. Regulation of lipoprotein metabolism by

thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. *Arterioscler Thromb Vasc Biol* 1997, 17:1756-1764.

Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptorγ (PPARγ). *J Biol Chem* 1995, 270:12953-12956.

Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N. Macrophage-induced angiogenesis is mediated by tumour necrosis factor-alpha. *Nature* 1987, 329:630-632.

Leininger MT, Portocarrero CP, Houseknecht KL. Peroxisome proliferator-activated receptor gamma1 expression in porcine white blood cells: dynamic regulation with acute endotoxemia. *Biochem Biophys Res Commun* 1999, 263:749-753.

Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK. Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J Clin Invest* 2000, 106:523-531.

Libby P, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation* 1997, 96:4095-4103.

Iijima K, Yoshizumi M, Ako J, Eto M, Kim S, Hashimoto M, Sugimoto N, Liang YQ, Sudoh N, Toba K, Ouchi Y. Expression of peroxisome proliferator-activated receptor gamma (PPAR gamma) in rat aortic smooth muscle cells. *Biochem Biophys Res Commun* 1998, 247:353-356.

Lindqvist P, Ostlund-Lindqvist AM, Witztum JL, Steinberg D, Little JA. The role of lipoprotein lipase in the metabolism of triglyceride-rich lipoproteins by macrophages. *J Biol Chem* 1983, 258:9086-9092.

Lin FT, Lane MD. Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes. *Genes Dev* 1992, 6:533-544.

Lin Q, Ruuska SE, Shaw NS, Dong D, Noy N. Ligand selectivity of the peroxisome proliferator-activated receptor alpha. *Biochemistry* 1999, 38:185-190.

Lipid Research Clinics Program. The lipid research clinics coronary primary prevention trial results: I. Reduction in incidence of coronary heart disease. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *J Am Med Ass* 1984, 251:351-374.

Lithell H, Boberg J, Hellsing K, Lundqvist G, Vessby B. Lipoprotein-lipase activity in human skeletal muscle and adipose tissue in the fasting and the fed states. *Atherosclerosis* 1978, 30:89-94.

Loskutoff DJ, Samad F. The adipocyte and hemostatic balance in obesity: studies of PAI-1. *Arterioscler Thromb Vasc Biol* 1997, 18:1-6.

Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986, 315:1046-1051.

Mach F, Schonbeck U, Fabunmi RP, Murphy C, Atkinson E, Bonnefoy JY, Graber P, Libby P. T lymphocytes induce endothelial cell matrix metalloproteinase expression by a CD40L-dependent mechanism: implications for tubule formation. *Am J Pathol* 1999, 154:229-238.

Maegawa H, Ide R, Hasegawa M, Ugi S, Egawa K, Iwanishi M, Kikkawa R, Shigeta Y, Kashiwagi A.Thiazolidine derivatives ameliorate high glucose-induced insulin resistance via the normalization of protein-tyrosine phosphatase activities. *J Biol Chem* 1995, 270:7724-7730.

Makoveichuk E, Lookene A, Olivecrona G. Mild oxidation of lipoproteins increases their affinity for surfaces covered by heparan sulfate and lipoprotein lipase. *Biochem Biophys Res Commun* 1998, 252:703-710.

Malaguarnera M, Giugno I, Ruello P, Vinci E, Panebianco MP, Motta M. Treatment of hypertriglyceridemia. Current aspects. *Recent Prog Med* 2000, 91:379-387.

Malinow MR, Nieto FJ, Szklo M, Chambless LE, Bond G. Carotid artery intimal-medial wall thickening and plasma homocysteine in asymptomatic adults: The Atherosclerosis Risk in Communities Study. *Circulation* 1993, 87:1107-1113.

Mamputu JC, Desfaits AC, Renier G. Lipoprotein lipase enhances human monocyte adhesion to aortic endothelial cells. *J Lipid Res* 1997, 38:1722-1729.

Marceau F. Evidence for vascular tone regulation by resident or infiltration leukocytes. *Biochem Pharmacol* 1996, 52:1481-1488.

Martin GA, Busch SJ, Meredith GD, Cardin AD, Blankenship DT, Mao SJT, Rechtin AE, Woods CW, Racke MM, Schafer MP, Fitzgerald MC, Burke DM, Flanagan MA, Jackson RL. Isolation and cDNA sequence of human postheparin plasma hepatic triglyceride lipase. *J Biol Chem* 1988, 263:10907-10914

Martin G, Schoonjans K, Lefebvre A, Staels B, Auwerx J. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPAR alpha and PPAR gamma activators. *J Biol Chem* 1997, 272:28210–28217.

Marx N, Bourcier T, Sukhova GK, Libby P, Plutzky J. PPARγ activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression. *Arterioscler Thromb Vasc Biol* 1999, 19:546-551.

Marx N, Schönbeck U, Lazar MA, Libby P, Plutzky J. Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. *Circ Res* 1999, 83:1097-1103.

Marx N, Sukhova G, Collins T, Libby P, Plutzky J. PPARα activators inhibit cytokine-induced vascular cell adhesion molecule-1 expression in human endothelial cells. *Circulation* 1999, 99:3125-3131.

Marx N, Sukhova GM, Murphy C, Libby P, Plutzky J. Macrophages in human atheroma contain PPAR gamma: Differentiation-dependent PPAR gamma expression and reduction of MMP-9 activity through PPAR gamma activation in mononuclear phagocytes in vitro. *Am J Pathol* 1998, 153:17-23.

Mascaro C, Acosta E, Ortiz J, Marrero P, Hegardt F, Haro D. Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. *J Biol Chem* 1998, 273:8560–8563.

Masuno H, Blanchette-Mackie EJ, Schultz CJ, Spaeth AE, Scow RO, Okuda H. Retention of glucose by N-linked oligosaccharide chains impedes expression of lipoprotein lipase activity: effect of castanospermine. *J Lipid Res* 1992, 33:1343-1349.

Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR. Menopause and risk factors for coronary heart disease. *N Engl J Med* 1989, 321:641-646.

Ma Y, Henderson HE, Liu MS, Zhang H, Forsythe IJ, Clarke-Lewis I, Hayden MR, Brunzell JD. Mutagenesis in four candidate heparin binding regions and identification of residues affecting heparin binding of human lipoprotein lipase. *J Lipid Res* 1994, 35:1511-1523.

Ma Y, Henderson HE, Ven Murthy V, Roederer G, Monsalve MV,Clarke LA, Normand T, Julien P, Gagné, C, Lambert M, Davignon J, Lupien PJ, Brunzell J, Hayden MR. A mutation in the human lipoprotein lipase gene as the most

common cause of familial chylomicronemia in French Canadians. *N Engl J Med* 1991, 324:1761-1766.

Mead JR, Cryer A, Ramji DP. Lipoprotein lipase, a key role in atherosclerosis? *FEBS Lett* 1999, 462:1-6.

Merkel M, Kako Y, Radner H, Cho IS, Ramasamy R, Brunzell JD, Goldberg IJ, Breslow JL. Catalytically inactive lipoprotein lipase expression in muscle of transgenic mice increases very low density lipoprotein uptake: direct evidence that lipoprotein lipase bridging occurs in vivo. *Proc Natl Acad Sci USA* 1998, 95:13841-13846.

McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. *Am J Pathol* 1996, 56:11-28.

McLenachan JM, Vita J, Fish DR, Treasure CB, Cox DA, Ganz P, Selwyn AP. Early evidence of endothelial vasodilator dysfunction at coronary branch points. *Circulation* 1990, 82:1169-1173.

Michael Pittilo R. Cigarette smoking, endothelial injury and cardiovascular disease. *Int J Exp Pathol* 2000, 81:219-230.

Michaud SE, Renier G. Direct regulatory effect of fatty acids on macrophage lipoprotein lipase: potential role of PPARs. *Diabetes* 2001, 50:660-666.

Minamikawa J, Tanaka S, Yamauchi M, Inoue D, Koshiyama H. Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes. *J Clin Endocrinol Metab* 1998, 83:1818-1820.

Moore KJ, Rosen ED, Fitzgerald ML, Randow F, Andersson LP, Altshuler D, Milstone DS, Mortensen RM, Spiegelman BM, Freeman MW. The role of PPAR-gamma in macrophage differentiation and cholesterol uptake. *Nat Med* 2001, 7:41-7.

Morikang E, Benson SC, Kurtz TW, Pershadsingh HA. Effects of thiazolidinediones on growth and differentiation of human aorta and coronary myocytes. *Am J Hypertens* 1997, 10:440-446.

Mori M, Yamaguchi K, Abe K. Purification of a lipoprotein lipase-inhibiting protein produced by a melanoma cell line associated with cancer cachexia. *Biochem Biophys Res Commun* 1989, 160:1085-1092.

Morohoshi M, Fujisawa K, Uchimura I, Numano F. Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes in vitro. *Diabetes* 1996, 45:954-959.

Morohoshi M, Fujisawa K, Uchimura I, Numano F. The effect of glucose and advanced glycosylation end products on IL-6 production by human monocytes. *Ann N Y Acad Sci* 1995, 748:562-570.

Motojima K, Passilly P, Peters J, Gonzalez F, Latruffe N. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. *J Biol Chem* 1998, 273:16710–16714.

Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, Andria G, Boers GH, Bromberg IL, Cerone R, et al. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet* 1985, 37:1-31.

Mulder M, Lombardi P, Jansen H, van Berkel TJ, Frants RR, Havekes Lm. Heparan sulphate proteoglycans are involved in the lipoprotein lipase-mediated enhancement of the cellular binding of very low density and low density lipoproteins. *Biochem Biophys Res Commun* 1992, 185:582-587.

Murao K, Imachi H, Momoi A, Sayo Y, Hosokawa H, Sato M, Ishida T, Takahara J. Thiazolidinedione inhibits the production of monocyte chemoattractant protein-1 in cytokine-treated human vascular endothelial cells. *FEBS Lett* 1999, 454:27-30.

Murata Y, Behr SR, Kraemer FB. Regulation of macrophage lipoprotein lipase secretion by the scavenger receptor. *Biochim Biophys Acta* 1988, 972:17-24.

Myklebost O, Williamson B, Markham AF, Myklebost SR, Rogers J, Woods DE, Humphries SE. The isolation and characterization of cDNA clones for human apolipoprotein CII. *J Biol Chem* 1984, 259:4401-4404.

Nagy L, Tontonoz P, Alvarez JGA, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. *Cell* 1998, 93:229-240.

Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL, Edwards PA, Fogelman AM. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. *Arterioscler Thromb Vasc Biol* 1996, 16:831-842.

Nehler MR, Taylor LM Jr, Porter JM. Homocysteinemia as a risk factor for *atherosclerosis: a review. Cardiovasc Surg* 1997, 6:559-567.

Neuzi J, Upston JM, Witting PK, Scott KF, Stocker R. Secretory phospholipase A2 and lipoprotein lipase enhance 16-lipoxygenase-induced enzymic and nonenzymic lipid peroxidation in low-density lipoprotiens. *Biochemistry* 1998, 37:9203-9210.

Newholmes P, Gordon S, Newholmes EA. Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages. *Biochem J* 1987, 9:161-167.

Nielsen LB, Juul K, Nordestgaard BG. Increased degradation of lipoprotein (a) in atherosclerotic compared with nonlesioned aortic intima-inner media of rabbits: in vivo evidence that lipoprotein (a) may contribute to foam cell formation. *Atheroscler Thromb Vasc Biol* 1998, 18:641-649.

Nieto FJ. Infections and atherosclerosis: new clues from an old hypothesis? *Am J Epidemiol* 1998, 148:937-948.

Nyard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997, 337:230-236.

Nykjaer A, Nielsen M, Lookene A, Meyer N, Roigaard H, Etzerodt M, Beisiegel U, Olivecrona G, Gliemann J. A carboxyl-terminal fragment of lipoprotein lipase bind to the low density lipoprotein receptor-related protein and inhibits lipase-mediated uptake of lipoprotein in cells. *J Biol Chem* 1994, 269:31747-31755.

O'Brien KD, Gordon D, Deeb S, Ferguson M, Chait A. Lipoprotein lipase is synthesized by macrophage-derived foam cells in human coronary atherosclerotic plaques. *J Clin Invest* 1992, 89:1544-1550.

O'Brien KD, Deeb SS, Ferguson M, McDonald TO, Allen MD, Alpers CE, Chait A. Apolipoprotein E localization in human coronary atherosclerotic plaques by in situ hybridization and immunohistochemistry and comparison with lipoprotein lipase. *Am J Pathol* 1994, 144:538-548.

Obunike JC, Paka S, Pillarisetti S, Goldberg IJ. Lipoprotein lipase can function as a monocyte adhesion protein. *Arterioscler Thromb Vasc Biol* 1997, 17:1414-1420.

Obunike JC, Pillarisetti S, Paka L, Kako Y, Butteri MJ, Ho YY, Wagner WD, Yamada N, Mazzone T, Deckelbaum RJ, Goldberg IJ. The heparin-binding

proteins apolipoprotein E and lipoprotein lipase enhance cellular proteoglycan production. *Arterioscler Thromb Vasc Biol* 2000, 20:111-118.

Ogawa H, Nielsen S, Kawakami M. Cachectin/tumor necrosis factor and interleukin-1 show different modes of combined effect on lipoprotein lipase activity and intracellular lipolysis in 3T3-L1 cells. *Biochim Biophys Acta* 1989, 1003:131-135.

Ohsumi J, Miyadai K, Kawashima I, Sakakibara S, Yamaguchi J, Itoh Y Regulation of lipoprotein lipase synthesis in 3T3-L1 adipocytes by interleukin-11/adipogenesis inhibitory factor. *Biochem Mol Biol Int* 1994, 32:705-712.

Olivecrona T, Chernick SS, Bengtsson-Olivecrona G, Garrison M, Scow RO. Synthesis and secretion of lipoprotein lipase in 3T3-L1 adipocytes. Demonstration of inactive forms of lipase in cells. *J Biol Chem* 1987, 262:10748-10759.

Ottosson M, Vikman-Adolfsson K, Enerback S, Elander A, Bjorntorp P, Eden S. Growth hormone inhibits lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* 1995, 80:936-941.

Pankow JS, Folsom AR, Province MA, Rao DC, Eckfeldt J, Heiss G, Shahar E, Wu KK. Family history of coronary heart disease and hemostatic variables in

middle-aged adults. Atherosclerosis Risk in Communities Investigators and Family Heart Study Research Group. *Thromb Haemost 1997*, 77:87-93.

Parkin SM, Speake BK, Robinson DS. Purification and characterization of rat adipose tissue lipoprotein lipase. *Biochem J* 1982, 207:485-495.

Patton JS, Shepard HM, Wilking H, Lewis G, Aggarwal BB, Eessalu TE, Gavin LA, Grunfeld C. Interferons and tumor necrosis factor have similar catabolic effects on 3T3 L1 cells. *Proc Natl Acad Sci USA* 1986, 83:8313-8317.

Paul SR, Bennette F, Calvetti JA, Kelleher K, Wood CR, O'Hara RM Jr, Leary AC, Sibley B, Clark SC, Williams DA, Yang YC. Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proc Natl Acad Sci USA* 1990, 87:7512-7516.

Pedersen SB, Borglum JD, Moller-Pedersen T, Richelsen B. Effects of in vivo estrogen treatment on adipose tissue metabolism and nuclear estrogen receptor binding in isolated rat adipocytes. *Mol Cell Endocrinol* 1992, 85:13-19.

Peters JM, Hennuyer N, Staels B, Fruchart JC, Fievet C, Gonzalez FJ, Auwerx J. Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor alpha-deficient mice. *J Biol Chem* 1997, 272:27307–27312.

Petrova TV, Akama KT, Van Eldik LJ. Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric oxide synthase by 15-deoxy-D 12,14-prostaglandin J2. *Proc Natl Acad Sci USA* 1999, 96:4668–4673.

Plutzky J. Peroxisome Proliferator-activated Receptors in Vascular Biology and Atherosclerosis: Emerging Insights for Evolving Paradigms. *Curr Atheroscler Rep* 2000, 2:327-335.

Posner I, Wang CS, McConathy WJ. The comparative kinetics of soluble and heparin-Sepharose-immobilized bovine lipoprotein lipase. *Arch Biochem Biophys* 1983, 226:306-316.

Pradines-Figueres A, Barcellini-Couget S, Dani C, Baudoin C, Ailhaud G. Inhibition by serum components of the expression of lipoprotein lipase gene upon stimulation by growth hormone. *Biochem Biophys Res Commun* 1990, 166:1118-1125.

Previato L, Parrott CL, Santamarina-Fojo S, Brewer HB. Transcriptional regulation of the human lipoprotein lipase gene in 3T3-L1 adipocytes. *J Biol Chem* 1991, 266:18958-18963.

Price SR, Mizel SB, Pekala PH. Regulation of lipoprotein lipase synthesis and 3T3-L1 adipocyte metabolism by recombinant interleukin 1. *Biochim Biophys Acta* 1986, 889:374-381.

Querfeld U, Ong JM, Prehn J, Carty J, Saffari B, Jordan SC, Kern PA. Effects of cytokines on the production of lipoprotein lipase in cultured human macrophages. *J Lipid Res* 1990, 31:1379-1386.

Ranganathan G, Vu D, Kern PA. Translational regulation of lipoprotein lipase by epinephrine involves a trans-acting binding protein interacting with the 3 untranslated region. *J Biol Chem* 1997, 272:2515-2519.

Ranganathan S, Kern PA. Thiazolidinediones inhibit lipoprotein lipase activity in adipocytes. *J Biol Chem* 1998, 273:26117-26122.

Rapp JH, Lespine A, Hamilton RL, Colyvas N, Chaumeton AH, Tweedie-Hardman J, Kotite L, Kunitake ST, Havel RJ, Kane JP. Triglyceride-rich lipoproteins isolated by selected-affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. *Arterioscler Thromb* 1994, 14:1767-1774.

Rath M, Niendorf A, Reblin T, Dietel M, Krebber HJ, Beisiegel U. Detection and quantification of lipoprotein(a) in the arterial wall of 107 coronary bypass patients. *Arteriosclerosis*. 1989, 9:579-592.

Renier G, Skamene E, Desanctis JB, Radzioch D. High macrophage lipoprotein lipase expression and secretion are associated in inbred murine strains with susceptibility to atherosclerosis. *Arterioscler Thromb.* 1993,13:190-196.

Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, Witztum JL, Auwerx J, Palinski W, Glass CK. Expression of the peroxisome proliferator-activated receptor gamma (PPAR gamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc Natl Acad Sci U S A* 1998, 95:7614-7619.

Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998, 391:79-82.

Robinson DS, Parkin SM, Speake BK, Little A. Hormonal control of rat adipose tissue lipoprotein lipase activity. In: The adipocyte and Obesity: Cellular and Molecular Mechanisms. A Angel, CH Hollenberg, DAK Roncar, eds. Raven, New York; 1983:127-136.

Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM. PPAR gamma is required for the differentiation of adipose tisue in vivo and in vitro. *Mol Cell* 1999, 4:611-617.

Rosenfeld ME, Ross R. Macrophage and smooth muscle cell proliferation in atheroclerotic lesions of WHHL and comparably hypercholesterolemic fat-fed rabbits. *Arteriosclerosis* 1990,10:680-687.

Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, Santoro MG. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. *Nature* 2000, 403:103-108.

Ross R. Atherosclerosis---an inflammation disease. *N Engl J Med* 1999, 340:115-126.

Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993, 362:80-89.

Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell: proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. *Science* 1973, 180:1332-1339.

Ross R. The pathogenesis of atherosclerosis--an update. *N Engl J Med* 1986, 314:488-500.

Rumsey SC, Obunike JC, Arad Y, Deckelbaum RJ, Goldberg IJ. Lipoprotein lipase-mediated uptake and degradation of low density lipoproteins by fibroblasts and macrophages. *J Clin Invest* 1992, 90:1504-1512.

Ruschitzka FT, Noll G, Luscher TF. The endothelium in coronary artery disease. *Cardiology* 1997, 88:3-19.

Rus HG, Niculescu F, Vlaicu R. Tumor necrosis factor-alpha in human arterial wall with atherosclerosis. *Atherosclerosis* 1991, 89:247-254.

Sadur CN, Yost TJ, Eckel RH. Insulin responsiveness of adipose tissue lipoprotein lipase is delayed but preserved in obesity. *J Clin Endocrinol Metab* 1984, 59:1176-1182.

Saitoh K, Mori TK, Kasai H, Nagayama T, Tsuchiya A, Ohbayashi S. Anti-atheromatous effects of fenofibrate, a hypolipidemic drug. I: Anti-atheromatous effects are independent of its hypolipidemic effect in cholesterol-fed rabbits. *Nippon Yakurigaku Zasshi* 1995, 106:41-50.

Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Makela PH, Huttunen JK, Valtonen V. Serological evidence of an association of a novel *Clamydia*, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet* 1998, 2:983-986.

Samuelsson L, Stromberg K, Vikman K, Bjursell G, Enerback S. The CCAAT/enhancer binding protein and its role in adipocyte differentiation: evidence for direct involvement in terminal adipocyte development. *EMBO J* 1991, 10:3787-3793.

Santamarina-Fojo S, Haudenschild C. Role of hepatic and lipoprotein lipase in lipoprotein metabolism and atherosclerosis: studies in transgenic and knockout animal models and somatic gene transfer. *Int J Tissue React* 2000, 22:39-47.

Sartippour MR, Lambert A, Laframboise M, St-Jacques P, Renier G. Stimulatory effect of glucose on macrophage lipoprotein lipase expression and production. *Diabetes.* 1998, 47:431-438.

Sartippour MR, Renier G. Differential regulation of macrophage peroxisome proliferator-activated receptor expression by glucose: role of peroxisome

proliferator-activated receptors in lipoprotein lipase gene expression. *Arterioscler Thromb Vasc Biol* 2000, 20:104-110.

Sartippour MR, Renier G. Upregulation of macrophage lipoprotein lipase in patients with type 2 diabetes: role of peripheral factors. *Diabetes* 2000, 49:597-602.

Satoh H, Tsukamoto K, Hashimoto Y, Hashimoto N, Togo M, Hara M, Maekawa H, Isoo N, Kimura S, Watanabe T. Thiazolidinediones suppress endothelin-1 secretion from bovine endothelial cells: a new possible role for PPARγ on vascular endothelial function. *Biochem Biophys Res Commun* 1999, 254:757-763.

Saxena U, Klein MG, Goldberg IJ. Identification and characterization of the endothelial cell surface lipoprotein lipase receptor. *J Biol Chem* 1991, 266:17516-17521.

Saxena U, Kulkarni NM, Ferguson E, Newton RS. Lipoprotein lipase-mediated lipolysis of very low density lipoproteins increases monocyte adhesion to aortic endothelial cells. *Biochem Biophys Res Commun* 1992, 189:1653-1658.

Schmidt C, Hulthe J, Wikstrand J, Gnarpe H, Gnarpe J, Agewall S, Fagerberg B. Chlamydia pneumoniae seropositivity is associated with carotid artery intima-media thickness. *Stroke* 2000, 31:1526-1531.

Schonbeck U, Mach F, Sukhova GK, Murphy C, Bonnefoy JY, Fabunmi RP, Libby P. Regulation of matrix metalloproteinase expression in human vascular smooth muscle cells by T lymphocytes: a role for CD40 signaling in plaque rupture? *Circ Res* 1997, 81:448-454.

Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J. PPAR alpha and PPAR gamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J* 1996, 15:5336-5348.

Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* 1996, 1302:93-109.

Schoonjans K, Staels B, Grimaldi P, Auwerx J. Acyl-CoA synthetase mRNA expression is controlled by fibric acid derivatives, feeding and liver proliferation. *Eur J Biochem* 1993, 216:615–622.

Schwartz SM, Gajdusek EM, Selden SC. Vascular wall growth control: the role of endothelium. *Arteriosclerosis* 1981, 1:107-161.

Semb H, Olivecrona T. The relation between glycosylation and activity of guinea pig lipoprotein lipase. *J Biol Chem* 1989, 264:4195-4200.

Semenkovich CF, Coleman T, Daugherty A. Effects of heterozygous lipoprotein lipase deficiency on diet-induced atherosclerosis in mice. *J Lipid Res* 1998, 39:1141-1151.

Semenkovich CF, Luo CC, Nakanishi MK, Chen SH, Smith LC, Chan L. In vitro expression and site-specific mutagenesis of the cloned human lipoprotein lipase gene. Potential N-linked glycosylation site asparagine 43 is important for both enzyme activity and secretion. *J Biol Chem* 1990, 265:5429-5433.

Serrero G, Lepak NM, Goodrich SP. Paracrine regulation of adipose differentiation by arachidonate metabolites: prostaglandin F2 alpha inhibits early and late markers of differentiation in the adipogenic cell line 1246. *Endocrinology* 1992, 131:2545-2551.

Shalve A, Siegrist-Kaiser CE, Yen PM et al. The peroxisome proliferator-

activated receptor α is a phosphoprotein: regulation by insulin. *Endocrinology* 1996, 137:4499-4502.

Shao D, Rangwala SM, Bailey ST, Krakow SL, Reginato MJ, Lazar MA. Interdomain communication regulating ligand binding by PPAR-gamma. *Nature* 1998, 396:377-380.

Shichiri M, Shinohara M, Suzaki K, Takeda H. Clinical significance of analysis of glycated lipoproteins in the diagnosis of diabetic angiopathies. *Nippon Rinsho* 1990, 48:334-340.

Shimada M, Shimano H, Gotoda T, Yamamoto K, Kawamura M, Inaba T, Yazaki Y, Yamada N. Overexpression of human lipoprotein lipase in transgenic mice. Resistance to diet-induced hypertriglyceridemia and hypercholesterolemia. *J Biol Chem* 1993, 268:17924-17929.

Shinohara E, Kihara S, Ouchi N, Funahashi T, Nakamura T, Yamashita S, Kameda-Takemura K, Matsuzawa Y. Troglitazone suppresses intimal formation following balloon injury in insulin-resistant Zucker fatty rats. *Atherosclerosis* 1998, 136:275-279.

Sianiko AR, Wells TG. Childhood hypertension. In: Hypertension, vol 2. Laragh JH, Brenner BM, eds. Raven, New York. 1990, 1855-1868.

Simsolo RB, Ong JM, Kern PA. Characterization of lipoprotein lipase activity, secretion, and degradation at different sites of post-translational processing in primary cultures of rat adipocytes. *J Lipid Res* 1992, 33:1777-1784.

Sivaram P, Klein MG, Goldberg IJ. Identification of a heparin-releasable lipoprotein lipase binding protein from endothelial cells. *J Biol Chem* 1992, 267:16517-16522.

Song J, Standley PR, Zhang F, Joshi D, Gappy S, Sowers JR, Ram JL. Tamoxifen (estrogen antagonist) inhibits voltage-gated calcium current and contractility in vascular smooth muscle from rats. *J Pharmacol Exp Ther* 1996, 277:1444-1453.

Sopher O, Goldman R. Bacterial lipopolysaccharide suppresses the expression of lipoprotein lipase in murine macrophages: a process independent of tumor necrosis factor or interleukin 1. *Immunol Lett* 1987, 15:261-265.

Spiegelman BM. PPAR γ in monocytes: less pain, any gain? *Cell* 1998, 93:153-155.

Spiegelman BM, Hu E, Kim JB, Brun R. PPAR gamma and the control of adipogenesis. *Biochimie* 1997, 79:111-112.

Staels B, Auwerx J. Perturbation of developmental gene expression in rat liver by fibric acid derivatives; lipoprotein lipase and α-fetoprotein as models. *Development* 1992, 115:1035-1043.

Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart J-C. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* 1998, 98:2088–2093.

Staels B, Koenig W, Habib A, Merval R, Lebret M, Torra IP, Delerive P, Fadel A, Chinetti G, Fruchart JC, Najib J, Maclouf J, Tedgui A. Activation of human aortic smooth muscle cells is inhibited by PPAR alpha but not by PPAR gamma activators. *Nature* 1998, 393:790-793.

Staels B, Vu-Dac N, Kosykh VA, Saladin R, Fruchart JC, Dallongeville J, Auwerx J. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. A potential mechanism for the hypolipidemic action of fibrates. *J Clin Invest* 1995, 95:705-712.

Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest* 1986, 77:1370-1376.

Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W jr, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1994, 89:2462-2478.

Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 1997, 272:20963-20966.

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989, 320:915-924.

Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized LDL. *Proc Natl Acad Sci USA* 1995, 92:3893-3897.

Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD. A novel therapy for colitis utilizing PPAR-γ ligands to inhibit the epithelial inflammatory response. *J Clin Invest* 1999, 104:383-389.

Sullivan GW, Sarembock IJ, Linden J. The role of inflammation in vascular diseases. *J Leukoc Biol* 2000, 67:591-602.

Tanaka T, Itoh H, Doi K, Fukunaga Y, Hosoda K, Shintani M, Yamashita J, Chun TH, Inoue M, Masatsugu K, Sawada N, Saito T, Inoue G, Nishimura H, Yoshimasa Y,Nakao K. Down regulation of peroxisome proliferator-activated receptor gamma expression by inflammatory cytokines and its reversal by thiazolidinediones. *Diabetologia* 1999, 42:702-710.

Taskinen MR, Nikkila EA. Effects of caloric restriction on lipid metabolism in man: changes of tissue lipoprotein lipase activities and of serum lipoproteins. *Atherosclerosis* 1979, 32:289-299.

Tengku-Muhammad TS, Hughes TR, Cryer A, Ramji DP. Differential regulation of lipoprotein lipase in the macrophage J774.2 cell line by cytokines. *Cytokine* 1996, 8:525-533.

Tengku-Muhammad TS, Hughes TR, Cryer A, Ramji DP. Involvement of both the tyrosine kinase and the phosphatidylinositol-3' kinase signal transduction

pathways in the regulation of lipoprotein lipase expression in J774.2 macrophages by cytokines and lipopolysaccharide. *Cytokine* 1999, 11:463-468.

Tengku-Muhammad TS, Cryer A, Ramji DP. Synergism between interferon gamma and tumour necrosis factor alpha in the regulation of lipoprotein lipase in the macrophage J774.2 cell line. *Cytokine* 1998, 10:38-48.

Thieringer R, Fenyk-Melody JE, Le Grand CB, Shelton BA, Detmers PA, Somers EP, Carbin L, Moller DE, Wright SD, Berger J. Activation of peroxisome proliferator-activated receptor gamma does not inhibit IL-6 or TNF-alpha responses of macrophages to lipopolysaccharide in vitro or in vivo. *J Immunol* 2000, 164:1046-1054.

Thompson SG, Kienase J, Pyke SDM, Haverkate F, van de Loo JCW. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N Engl J Med* 1995, 332:635-641.

Tintut Y, Patel J, Parhami F, Demer LL. Tumor necrosis factor-alpha promotes in vitro calcification of vascular cells via the cAMP pathway. *Circulation* 2000, 102:2636-2642.

Tontonoz P, Nagy L, Alvarez J, Thomazy V, Evans R. PPAR γ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* 1998, 93: 241-252.

Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, Lee ME. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci USA* 1994, 91:6369-6373.

Tsutsumi K, Inoue Y, Hagi A, Murase T. The novel compound NO-1886 elevates plasma high-density lipoprotein cholesterol levels in hamsters and rabbits by increasing lipoprotein lipase without any effect on cholesteryl ester transfer protein activity. *Metabolism* 1997, 46:257-260.

Tsutsumi K, Inoue Y, Shima A, Murase T. Correction of hypertriglyceridemia with low high-density lipoprotein cholesterol by the novel compound NO-1886, a lipoprotein lipase-promoting agent, in STZ-induced diabetic rats. *Diabetes* 1995, 44:414-417.

UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998, 352:837-853.

Umek RM, Friedman AD, McKnight SL. CCAAT-enhancer binding protein: a component of a differentiation switch. *Science* 1991, 251:288-292.

Utermann G. Lipoprotein(a). In: Scriver CR, Beaudet AL, Sly WSL, eds. The Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill, New York. 1995, 1887-1912.

Uyemura K, Demer L, Castle SC et al. Cross-regulatory roles of interleukin (IL)-12 and IL-10 in atherosclerosis. *J Clin Invest* 1996, 97:2130-2138.

Van De Graaff, K.M. & Fox, I.S. Concepts of Human Anatomy and Physiology. WCB Publishers. U.S.A. *Circulatory System* 1992, 544-582.

Van Eck M, Zimmermann R, Groot PH, Zechner R, Van Berkel TJ. Role of macrophage-derived lipoprotein lipase in lipoprotein metabolism and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2000, 20:53-62.

Vannier C, Etienne J, Ailhaud G. Intracellular localization of lipoprotein lipase in adipose cells. *Biochim Biophys Acta* 1986, 875:344-354.

Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, Vekshtein VI, Selwyn AP, and Ganz P. Coronary vasomotor response to

acetylcholine relates to risk factors for coronary artery disease. *Circulation* 1990, 81:491-497.

Vu-Dac N, Schoonjans K, Laine B, Fruchart J, Auwerx J, Staels B. Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element . *J Biol Chem* 1994, 269:31012-31018.

Wall RT, Harlan JM, Harker LA, Striker GE. Homocysteine-induced endothelial cell injury *in vitro:* a model for the study of vascular injury. *Thromb Res* 1998, 18:113-121.

Wang X, Greilberger J, Jurgens G. Calcium and lipoprotein lipase synergistically enhance the binding and uptake of native and oxidized LDL in mouse peritoneal macrophages. *Atherosclerosis* 2000, 150:357-363.

Watanabe N, Niitsu Y, Umeno H, Kuriyama H, Neda H, Yamauchi N, Maeda M, Urushizaki I. Toxic effect of tumor necrosis factor on tumor vasculature in mice. *Cancer Res* 1988, 48:2179-2183.

Watanabe T, Fan JL. Atherosclerosis and inflammation---Mononuclear cell recruitment and adhesion molecules with reference to the implication of ICAM-1/LFA-1 pathway in atherogenesis. *Int J Cardiol* 1998, 66:45-53.

Welch GN, Upchurch GR Jr, Loscalzo J. Homocysteine, oxidative stress, and vascular disease. *Hosp Pract* 1997, 32:88-92.

White Jr, chait A, Klebanoff SJ, Deeb S, Brunzell JD. Bacterial lipopolysaccharide reduces macrophage lipoprotein lipase levels: an effect that is independent of tumour necrosis factor. *J Lipid Res* 1988, 29:1379-1385.

Wick G, Schett G, Amberger A, Kleindienst R, Xu Q. Is atherosclerosis an immunologically mediated disease? *Immunol Today* 1995, 16:27-33.

Wilensky RL, March KL, Gradus-Pizlo K, Sandusky G, Fineberg N, Hathaway DR. Vascular injury, repair, and restenosis after percutaneous transluminal angioplasty in the atherosclerotic rabbit. *Circulation* 1995, 92:2995-3005.

Williams KJ, Fless GM, Petrie KA, Snyder ML, Brocia RW, Swenson TL. Mechanisms by which lipoprotein lipase alters cellular metabolism of lipoprotein(a), low density lipoprotein, and nascent lipoproteins. Roles for low density lipoprotein receptors and heparan sulfate proteoglycans. *J Biol Chem* 1992, 267:13284-13292.

Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol* 1995, 15:551-561.

Willson TM, Lehmann JM, Kliewer SA. Discovery of ligands for the nuclear peroxisome proliferator-activated receptors. *Ann N Y Acad Sci* 1996, 804:276-283.

Winkler FK, D'Arcy A, Hunziker W. Structure of human pancreatic lipase. *Nature* 1990, 343:771-774.

Wong H, Davis RC, Nikazy J, Seebart KE, Schotz MC. Domain exchange: characterization of a chimeric lipase of hepatic lipase and lipoprotein lipase. *Proc Natl Acad Sci USA* 1991, 88:11290-11294.

Xing H, Northrop JP, Grove JR, Kilpatrick KE, Su JL, Ringold GM. TNF alpha-mediated inhibition and reversal of adipocyte differentiation is accompanied by suppressed expression of PPAR gamma without effects on Pref-1 expression. *Endocrinology* 1997, 138:2776-2783.

Xin X, Yang S, Kowalski J, Gerritsen ME. Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis in vitro and in vivo. *J Biol Chem* 1999, 274:9116-9121.

Yagyu H, Ishibashi S, Chen Z, Osuga J, Okazaki M, Perrey S, Kitamine T, Shimada M, Ohashi K, Harada K, Shionoiri F, Yahagi N, Gotoda T, Yazaki Y,

Yamada N. Overexpressed lipoprotein lipase protects against atherosclerosis in apolipoprotein E knockout mice. *J Lipid Res* 1999, 40:1677-1685.

Yarnell JW, Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ, Elwood PC. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. *Circulation* 1991, 83:836-844.

Yin B, Loike JD, Kako Y, Weinstock PH, Breslow JL, Silverstein SC, Goldberg IJ. Lipoprotein lipase regulates Fc receptor-mediated phagocytosis by macrophage maintained in glucose-deficient medium. *J Clin Invest.* 1997, 100:649-657.

Yla-Herttuala S, Lipton BA, Rosenfeld ME, Goldberg IJ, Steinberg D, Witztum JL. Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic lesions. *Proc Natl Acad Sci USA* 1991, 88:10143-10147.

Zhang B, Berger J, Hu E, Szalkowski D, White-Carrington S, Spiegelman BM, Moller DE. Negative regulation of peroxisome proliferator-activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-alpha. *Mol Endocrinol* 1996, 10:1457-1466.

Zhang F, Sowers JR, Ram JL, Standley PR, Peuler JD. Effects of pioglitazone on calcium channels in vascular smooth muscle. *Hypertension* 1994, 24:170-175.

Zhou YC, Waxman DJ. Cross-talk between Janus Kinase-Signal Transducer Activator of Transcription (JAK-STAT) and peroxisome proliferator-activated receptorα (PPAR α) signaling pathways. *J Biol Chem* 1999, 274:2672-2681.

Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation* 1979, 60:473-85.

Zimmermann R, Sartipy P, Winkler R, Zechner R, Hurt-Camejo E, Kostner GM. Endogenously produced glycosaminoglycans affecting the release of lipoprotein lipase from macrophages and the interaction with lipoproteins. *Biochim Biophys Acta* 2000, 1484:316-324.

Zsigmond E, Kobayashi K, Tzung KW, Li L, Fuke Y, Chan L. Adenovirus-mediated gene transfer of human lipoprotein lipase ameliorates the hyperlipidemias associated with apolipoprotein E and LDL receptor deficiencies in mice. *Hum Gene Ther* 1997, 8:1921-1933.