Peroxisome Proliferator-Activated Receptors and Liver X Receptors in Atherosclerosis and Immunity\textsuperscript{1,2}

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ABSTRACT Atherosclerosis is a primary cause of death in the United States, and the current obesity epidemic threatens to exacerbate its morbidity and mortality worldwide. Despite important cardiovascular treatment advances over the past few decades, new approaches are needed to curb dangerous health trends. Nuclear receptors are a superfamily of ligand-activated transcription factors. The discovery of subfamilies known as peroxisome proliferator-activated receptors (PPAR) and liver X receptors (LXR) as lipid-sensors that regulate lipid and glucose metabolism as well as inflammation offers new targets for nutritional and pharmacologic treatment of cardiovascular disease.

KEY WORDS: • peroxisome proliferator-activated receptors • liver X receptors • atherosclerosis

Cardiovascular disease reigns as the leading cause of death in the Western world. In the United States alone, it claimed the lives of nearly 1 million people in 2002, with >70\% of these deaths attributed to coronary heart disease or stroke (1). The future may be even bleaker; the current epidemic of obesity and its associated problems of insulin resistance, diabetes, dyslipidemia, hypertension, and atherosclerosis are projected by some to decrease the average lifespan over the next century (2). Collectively, these disorders are known as the metabolic syndrome (or syndrome X) and represent one of the greatest worldwide economic, social, and medical challenges that we must face. Indeed, globalization and the spread of the American diet to the developing world has paralleled explosive increases in the rates of obesity across the Pacific Rim, India, and other regions, where cardiovascular disease is similarly now burgeoning (1).

Over the past decade, significant strides were made in elucidating the molecular and cellular basis of atherosclerosis. This led to the classic “diet-heart” hypothesis that dietary fats increase serum cholesterol levels and that increased serum cholesterol levels predict cardiovascular disease in humans. This model has proven to be oversimplified because several clinical studies have failed to validate it. However, newer studies including the Lyon Diet Heart Study indicate that nutritional inputs such as the relative amounts of trans-fats, saturated fats, and polyunsaturated fats may affect cardiovascular morbidity and mortality (7).

A diet-nuclear receptor-heart hypothesis? A connection between nutrition and cardiovascular disease has long been postulated. In the 1950s it was shown that diets rich in fat increase serum cholesterol levels and that increased serum cholesterol levels predict cardiovascular disease in humans. This led to the classic “diet-heart” hypothesis that dietary fats and cholesterol may be primary causes of atherosclerosis (5,6). This model has proven to be oversimplistic because several clinical studies have failed to validate it. However, newer studies such as the Lyon Diet Heart Study indicate that nutritional inputs such as the relative amounts of trans-fats, saturated fats, and polyunsaturated fats may affect cardiovascular morbidity and mortality (7).

\textsuperscript{1} Published in a supplement to \textit{The Journal of Nutrition}. Presented at the “Nutrients, Nuclear Receptors, Inflammation, and Immunity” symposium held April 3, 2005 at Experimental Biology 2005 in San Diego, California. The symposium was sponsored by the National Institutes of Health Office of Dietary Supplements, the U.S. Department of Agriculture, Wyeth Nutrition, and the American Society for Nutrition. The symposium was chaired by Charles Stephensen of the U.S. Department of Agriculture Western Human Nutrition Research Center at the University of California, Davis, and by Margherita Cantorna of the Nutrition Department at the Pennsylvania State University.

\textsuperscript{2} G.D.B. is supported by the UCSD Institute of Molecular Medicine and a National Institutes of Health Kirschstein-National Research Service Award #1F32DK071478.

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0022-3166/06 $8.00 © 2006 American Society for Nutrition.
The interplay between diet and disease, as suggested by epidemiologic studies, has been bolstered by molecular advances in cardiovascular research. Peroxisome proliferator-activated receptors (PPAR) and liver X receptors (LXR) are lipid-sensing nuclear receptors and represent a nexus among nutrition, metabolism, and inflammation. They contain DNA and ligand binding domains that typify other members of the nuclear receptor superfamily, such as the glucocorticoid, estrogen, and androgen receptors. PPAR and LXR function as obligate heterodimers with retinoid X receptors (RXR) and influence gene transcription through multiple mechanisms (8). PPAR-RXR or LXR-RXR heterodimers bind to conserved DNA motifs containing tandem repeats of hexameric sequences separated by 1 bp (DR1) or 4 bp (DR4), respectively. In the absence of a ligand, these DNA-bound heterodimers can recruit complexes of proteins including the co-repressors silencing mediator of retinoid and thyroid receptors and/or nuclear receptor co-repressor, histone deacetylases, and chromatin-modifying proteins that actively repress transcription (9,10). With the addition of a ligand, heterodimeric receptors undergo a conformational shift, resulting in the release of corepressor complexes, the binding of coactivator complexes, and the recruitment of RNA polymerase II to activate gene expression. Ligand-bound receptors may also inhibit other signal transduction pathways, such as nuclear factor-kB (NF-kB), via protein-protein interactions that are independent of binding to DNA. Unlike classical endocrine receptors that bind with high affinity to glandular hormones, PPAR and LXR bind at lower, generally micromolar affinities to ligands generated from dietary sources or intracellular metabolism. To date, several lipids were suggested to be naturally occurring ligands for PPAR or LXR, including PUFA and prostaglandin metabolites or oxysterols, respectively. In keeping with their functions as lipid sensors, ligand-bound PPAR or LXR activate feed-forward metabolic cascades that regulate lipid homeostasis via the transcription of genes involved in lipid metabolism, storage, and transport (8).

The LXR subfamily is composed of 2 receptor isoforms, LXRα and LXRβ, which direct systemic cholesterol metabolism (8). The PPAR subfamily consists of 3 receptor isoforms, i.e., α, γ, and δ (also known as β), each with overlapping but distinct expression patterns and functions (11). Together, the PPAR regulate diverse aspects of fat metabolism through their coordinated activities in muscle, adipose tissue, and the liver. Notably, members of both the LXR and PPAR subfamilies are expressed in foam cell macrophages, where they control fat and cholesterol metabolism but also regulate inflammation (12,13). Their combined effects on peripheral metabolism, foam cell lipid homeostasis, and vascular wall inflammation have made the LXR and PPAR major targets in the treatment of obesity, diabetes, and cardiovascular disease.

LXR. LXR regulate whole-body cholesterol metabolism and bind to physiologic levels of cholesterol metabolites including 25-hydroxycholesterol and 24,25-epoxycholesterol (8). The LXRα isoform is found primarily in fat, liver, and intestinal tissue as well as macrophages, whereas LXRβ is broadly expressed. Both LXR regulate cholesterol metabolism through their coordinated control of genes involved in cholesterol transport and bile and fatty acid synthesis. Examples of LXR target genes include the ATP-binding cassette subfamilies, ABCA1 and ABCG1 (involved in cholesterol efflux), ABCG5 and ABCG8 (regulators of bile acid excretion and intestinal cholesterol absorption), sterol regulatory element binding protein, fatty acid synthase, sterol CoA desaturase 1, and acyl CoA carboxylase (factors that drive lipogenesis) (12). Within the foam cell macrophage, LXR regulate reverse cholesterol transport via their transcriptional control of ABCA1 and ABCG1, removing cholesterol from the vascular wall (12,14). In addition, LXR inhibit inflammation by antagonizing NF-kB signaling (13). Interestingly, LXRα, but not LXRβ, was implicated recently in macrophage-mediated immunity to infection by Listeria monocytogenes; this appeared to be driven by LXRα’s unique transcriptional activation of the antiapoptotic factor Sper apoptosis inhibitor in macrophage 1 (15,16). Systemic administration of LXR agonists to atherosclerosis-prone mice lowers LDL cholesterol (LDL-C), raises HDL cholesterol (HDL-C), and diminishes vascular lesion size (17,18). However, these compounds also result in hepatic fat synthesis and hypertriglyceridemia, which has limited their development as clinical drugs (19).

PPARα. PPARα is expressed primarily in the brown fat and liver, where it regulates genes involved in fatty acid oxidation, including fatty acid binding protein, acyl-CoA oxidase, and cytochrome P450 (11,20). Fibric acid derivatives, including fenofibrate and gemfibrozil, activate PPARα and ameliorate hypertriglyceridemia in humans. The role of PPARα in the foam cell has been difficult to ascertain because it is expressed in human but not mouse macrophages. Nevertheless, it was suggested that PPARα may promote apolipoprotein AI–dependent cholesterol efflux from macrophages and also have anti-inflammatory effects within the vascular wall (21,22). Indeed, studies in humans, such as the VA HIT trial, demonstrated that PPARα agonists increase HDL-C levels and improve cardiovascular disease outcomes (23).

PPARγ. A decade ago, PPARγ was identified as the molecular target of insulin-sensitizing drugs known as thiazolidinediones, spawning tremendous research efforts to understand its functions (24,25). PPARγ is expressed in several key metabolic tissues including skeletal muscle, liver, and most abundantly, in fat. In fat, it is essential for normal tissue development, and genetic deletion or dominant-negative mutations of PPARγ result in lipodystrophy and insulin resistance (26–30). Tissue-specific knockout studies in mice demonstrate that skeletal muscle and liver are similarly involved in the regulation of glucose homeostasis by PPARγ (31–34). PPARγ activates a battery of genes involved in lipid storage and lipogenesis, allowing adipose tissue to safely store greater quantities of fat and thereby reduce serum free fatty acids and the ectopic deposition of triglycerides in liver and muscle (11). In addition, PPARγ upregulates the expression of the fat-derived hormone adiponectin, which promotes insulin sensitivity, and represses fat expression of resistin and tumor necrosis factor-α, which cause insulin resistance (11). In addition to its important role in glucose homeostasis, PPARγ is expressed in the foam cell macrophage and has direct effects on the vascular wall. PPARγ enhances foam cell cholesterol uptake by upregulating the scavenger receptor CD36 but conversely promotes cholesterol efflux via upregulation of ABCG1 and indirectly through upregulation of LXRα (21,35–37). Furthermore, PPARγ ligands have anti-inflammatory effects on the macrophage, although some of these appear to be receptor independent (38–41). Evidence from both receptor loss-of-function studies and ligand treatment studies consistently suggests that PPARγ and its activators protect against atherogenesis (37,42–45).

PPARδ. PPARδ was once considered the most enigmatic of the PPAR, but recent studies have identified it as an important regulator of lipid metabolism, energy expenditure, and atherosclerosis (11). PPARδ occurs broadly, including...
expression in adipose tissue, muscle, liver, heart, skeletal muscle, and macrophages. Treatment of obese monkeys with a synthetic PPARγ activator, GW501516, produced dramatic changes in metabolic variables, including a 79% increase in serum HDL-C levels, a 56% decrease in triglycerides, a 29% decrease in LDL-C, and a 48% decrease in fasting insulin levels (46). This report, along with gain- and loss-of-function studies performed in our laboratory and elsewhere have heightened interest in PPARγ as a key metabolic and cardiovascular regulator (9,47–49).

Using a genetic gain-of-function approach, mice harboring transgenes of PPARγ fused to a viral protein (VP16 ligand-independent transactivation domain (VP16-PPARγ)) were created to explore the role for this receptor in fat and muscle. Mice expressing VP16-PPARγ selectively in adipose tissue, under the control of the enhancer-promoter of the adipocyte fatty acid binding protein gene, have dramatically reduced fat stores, reduced numbers and sizes of lipid droplets in their adipocytes, and decreased serum triglycerides and free fatty acid binding protein gene, have dramatically reduced fat under the control of the enhancer-promoter of the adipocyte fatty acid binding protein gene, have dramatically reduced fat stores, reduced numbers and sizes of lipid droplets in their adipocytes, and decreased serum triglycerides and free fatty acids (48). Moreover, transgenic mice weigh significantly less than controls, when fed either a standard laboratory diet or a high-fat diet, despite having comparable food intake. Adipose-specific expression of VP16-PPARγ in genetically obese leptin receptor db/db-null mice also protects against weight gain. These effects are due to the activation of genes involved in energy uncoupling and fatty acid oxidation, including uncoupling proteins 1 and 3, long- and very-long-chain fatty acyl-CoA synthetase, acyl CoA oxidase, carnitine palmitoyltransferase-1, and long- and very-long-chain acyl-CoA dehydrogenase. A second transgenic mouse line expressing VP16-PPARγ under the control of the muscle-specific, α-skeletal actin promoter was also developed (49). Interestingly, these mice had increased numbers of oxidative, type 1 muscle fibers, were resistant to obesity, and had enhanced running endurance. Importantly, synthetic PPARγ agonists similarly upregulated the expression of energy uncoupling and fatty acid oxidation genes in adipose tissue and muscle and protect against obesity (48,49). These findings raise hope that PPARγ synthetic agonists may be useful as therapeutics for obesity by reprogramming metabolism.

To investigate the role of PPARγ in cardiovascular disease, PPARγ-deficient bone marrow was transplanted into atherosclerosis-prone LDL receptor-null mice (9). After 8 wk of consuming an atherogenic diet, these mice were compared with similarly treated mice transplanted with wild-type bone marrow. Surprisingly, recipients of the PPARγ-null marrow had lesions that were reduced by >50% relative to wild-type transplanted controls. PPARγ-null macrophages revealed no differences in cholesterol export, nor did transplanted mice have altered serum cholesterol profiles relative to wild-type controls. However, PPARγ−/− macrophages expressed decreased levels of inflammatory mediators including monocyte chemoattractant protein-1, IL-1β, and matrix metalloproteinase-9. Conversely, macrophages overexpressing PPARγ expressed increased levels of inflammatory markers. Importantly, treatment of wild-type macrophages with synthetic PPARγ ligands suppressed inflammation, mimicking the reduced inflammation found in PPARγ-null cells. Binding studies revealed that PPARγ interacts with the inflammatory suppressor protein B-cell CLL/lymphoma 6 in a ligand-dependent manner. Hence, genetic deletion of PPARγ or ligation of PPARγ with receptor agonists liberates a negative regulator of inflammation, thereby producing anti-inflammatory effects. These findings suggest that PPARγ agonists may be therapeutic in the treatment of atherosclerosis.

CONCLUSIONS

Studies over the past decade identified key roles for LXR and PPAR in systemic lipid and glucose metabolism, energy homeostasis, and inflammatory control. As receptors for lipids, these transcription factors provide molecular pathways by which diet, like synthetic drugs, could affect disorders including dyslipidemia, diabetes, obesity, and cardiovascular disease. Questions still remain, however, concerning how best to harness their therapeutic potentials. Could we, for instance, make dietary choices to enhance PPAR activity? The (n-3) PUFA, such as eicosapentaenoic acid and docosahexaenoic acid, and the (n-6) PUFA, such as linoleic acid, and their metabolites are suggested to bind and activate PPAR (50–53). However, these reports are based largely on in vitro studies utilizing concentrations that may be substantially higher than physiologic levels of such fatty acids (54,55). Work to determine whether dietary manipulations can enhance concentrations of putative ligands to levels that are of functional significance in vivo is warranted. Moreover, studies using knock-out mice to determine whether the biological effects of dietary lipids are genetically dependent upon PPAR or LXR will be critical to fortify the link between diet, nuclear receptors, and modulation of disease.

Questions also remain about the utility of PPAR and LXR drugs in the treatment of atherosclerosis. Pharmacologic activators of PPAR, to date, are the only proven PPAR or LXR ligands to improve cardiovascular disease outcomes in humans (23,56). Studies to formally address the efficacy of PPARγ agonists in atherosclerosis are ongoing, but their therapeutic benefit is uncertain at this time. Early clinical evidence is favorable, however, based upon reductions in serum markers for atherosclerosis and carotid arterial wall thickness with thiazolidinedione treatment (57,58). PPARγ ligands, in studies done in our laboratory and elsewhere, appear promising as therapeutics for obesity and atherosclerosis. However, these agents are not currently approved for use in humans, and their safety as clinical drugs is unknown. Similarly, synthetic LXR drugs are unavailable for clinical use, and it is unclear whether new compounds can be designed to separate the adverse side effects of current drugs, including hypertriglyceridemia and steatosis, from the beneficial effects on cholesterol metabolism and inflammation. Basic and clinical investigations promise to shed light on these areas of uncertainty in the years to come.

ACKNOWLEDGMENTS

I thank Ronald Evans and all other members of the Evans laboratory, particularly Yong-Xu Wang and Chih-Hao Lee, for their illuminating studies on the functions of PPARγ.

LITERATURE CITED

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