Peroxisome proliferator-activated receptor gamma (PPAR-γ) and its effects on the Cardiovascular system

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Abstract
Cardiovascular disease is one of the leading causes of death in the United States. Studying the pathogenesis of cardiovascular disease is, therefore, essential for the possible alleviation of such cases. Further knowledge could lead to more effective drug treatment. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a nuclear receptor found in vascular-associated cells such as endothelial cells, macrophages, and vascular smooth muscle cells. Its role in cardiovascular pathogenesis has garnered great interest in recent years. Nonsense mutation studies show that PPAR-γ is essential for normal vascular function. Some studies even suggest that increased activation of PPAR-γ improves vascular function in dysfunctional states such as hypertension. However, some studies question PPAR-γ’s role and even suggest that it may play a negative role in some cardiovascular diseases, such as atherosclerosis. The overall evidence currently suggests that PPAR-γ is a potential target for agonists in combating cardiovascular disease, yet it is still unclear whether or not PPAR-γ has potentially negative effects on cardiovascular health when it is up-regulated.

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Cardiovascular disease is one of the leading causes of death in the United States. Studying the pathogenesis of cardiovascular disease is, therefore, essential for the possible alleviation of such cases. Further knowledge could lead to more effective drug treatment. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a nuclear receptor found in vascular-associated cells such as endothelial cells, macrophages, and vascular smooth muscle cells. Its role in cardiovascular pathogenesis has garnered great interest in recent years. Nonsense mutation studies show that PPAR-γ is essential for normal vascular function. Some studies even suggest that increased activation of PPAR-γ improves vascular function in dysfunctional states such as hypertension. However, some studies question PPAR-γ’s role and even suggest that it may play a negative role in some cardiovascular diseases, such as atherosclerosis. The overall evidence currently suggests that PPAR-γ is a potential target for agonists in combating cardiovascular disease, yet it is still unclear whether or not PPAR-γ has potentially negative effects on cardiovascular health when it is up-regulated.

**Topic:** What are the causes and mechanisms of cardiovascular disease and what is the function of PPAR-γ protein in the cardiovascular system?

**General Background:**

The human cardiovascular system is the body’s network of vessels and cardiac chambers used for conducting blood (Caterina and Libby, 2007), the fluid responsible for the transport of oxygen, nutrients, chemical messenger molecules, and waste products throughout the entire body. Regulation of the cardiovascular system is extremely important in order to maintain homeostasis and overall health. Cardiovascular disease is one of the leading causes of death in the U.S. and is predicted to rise to the top of diseases causing mortality world-wide within this century (Caterina and Libby, 2007; Braunwald *et al*., 2007). Accordingly, it is necessary to study the mechanisms that regulate the cardiovascular system. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a receptor protein located in the nucleus of all cells and is involved in a multitude of physiologically relevant molecular pathways throughout various body systems.
Three of the most noteworthy cells that PPAR-γ proteins are expressed in are endothelial cells, vascular smooth muscle cells, and cardiomyocytes, all cells primarily composing the cardiovascular system. This indicates the possibility that PPAR-γ has a function in the regulation of cardiovascular physiology and, therefore, may be involved in the pathogenesis of cardiovascular diseases.

The endothelium of the cardiovascular system is what comprises the interior layer of vasculature walls that function as a barrier between the blood and the rest of the body (Marx et al., 1999). The vascular endothelial cells are responsible for maintenance of blood pressure, vascular tone, and leukocyte movement (Caterina and Libby, 2007). Regulation of endothelial cells is done via secretions of various cytokines, enzymes, and other molecules, such as nitrous oxide and plasminogen activator inhibitor type-1 (PAI-1). PAI-1 is a serine protease inhibitor and functions in the regulation of blood clot formation, preventing the formation of life-threatening clots (Marx et al., 1999). PAI-1 is secreted from endothelial cells and when not regulated, may be involved in vascular dysfunction such as thrombosis (Wiman and Hamsten, 1991), a dangerous venous blood clot.

Beyond the endothelial cells is a basement membrane produced by vascular smooth muscle cells (Braunwald et al., 2007). These smooth muscle cells are required for the adjustment of arteriole diameter; therefore, regulating blood pressure (Rensen et al., 2007). This is in conjunction with the baroreflex, which is one of the body’s mechanisms for detecting changes in blood pressure and for making the appropriate modifications. Baroreceptors, or pressure sensors, in the aortic and carotid arteries sense blood pressure changes and then send signals to the brain, which in turn causes parasympathetic nervous system activation or inhibition and sympathetic nervous system activation or inhibition (Lanfranchi and Somers, 2002). The vascular smooth
muscle cells respond by altering arteriole diameter. It is apparent that dysfunction of either vascular endothelial cells or smooth muscle cells can lead to cardiovascular dysfunction.

As stated previously, cardiovascular disease is one of the leading causes of death in the U.S. and, therefore, is of high importance in the medical research field. Cardiovascular diseases include dysfunctions of the vasculature such as inflammation, hypertension, hypertrophy, thrombosis, and atherosclerosis (Duan et al., 2008; Beyer et al., 2008a; Beyer et al., 2008b). Risk factors for cardiovascular disease include smoking, high levels of cholesterol, diabetes, a high fat diet, and obesity (Duan et al., 2008; Zhao et al., 2012). These factors all contribute to damage of the vasculature.

For instance, obesity has been shown to be correlated with the increased progression of atherosclerosis in men (McGill et al., 2002), as well as with decreased baroreflex function (Zhao et al., 2012). Atherosclerosis is the formation of fatty streaks that become full of various cells including leukocytes, endothelial cells, platelets, and vascular smooth muscle cells (Plutzky, 2003). This plaque can harden and increase in size, causing a decrease in blood flow. With this increase in size comes a decrease in diameter around the plague. If a blood clot moves through this section of vessel, it can become lodged, hindering or potentially stopping blood flow. One of the driving forces behind atherosclerosis formation is inflammation (Plutzky, 2003). In order to further understand how to combat cardiovascular diseases, such as atherosclerosis, the pathways of molecular mediators involved in inflammation must be investigated. One possible mediator of interest is PPAR-γ.

The PPAR protein family consists of three hormone receptors located in the cell nucleus: α, β/δ, and γ (Kulkarni et al., 2012; Ryan et al., 2004). The roles of these PPAR receptors vary, but it has been shown that they are involved in the regulatory pathways of inflammation,
immunity, cell proliferation and differentiation, metabolism, adipogenesis, blood pressure, and apoptosis (Duan et al., 2008; Kulkarni et al., 2012; Ryan et al., 2004; Ketsawatsomkron et al., 2010; Hamblin et al., 2010). These receptors are activated via ligand binding. Ligands that activate PPAR receptors include fatty acids, prostaglandins, and members of the drug family thiazolidinedones (TZDs) (Tsai et al., 2004). Some authors consider PPAR receptors to be lipid sensors, affecting various regulatory pathways based on blood lipid levels (Kulkarni et al., 2012).

One of the PPAR family members, PPAR-γ, has been of upmost interest in the research literature as important for having a role in adipogenesis, blood pressure, endothelial function, and the inflammatory response. PPAR-γ protein has two main isoforms: PPAR-γ1 and PPAR-γ2. Both isoforms appear to be involved in similar pathways, with little difference in ligand specificity. PPAR-γ2 is expressed in adipocytes only and PPAR-γ1 is expressed in all other cells (Rangwala and Lazar, 2004), most notably endothelial cells, vascular smooth muscle cells, and even macrophages (Duan et al., 2008; Hamblin et al., 2010). Therefore, it seems vital to investigate the role of PPAR-γ proteins in various vascular diseases.

Once ligand activation of PPAR-γ occurs, retinoid x receptor (RXR) binds to form a heterodimer with PPAR-γ, thus causing the release of the previously present co-repressors blocking PPAR-γ activity (Duan et al., 2008; Kulkarni et al., 2012). This then allows for co-activators to bind and for transcription of specific targeted genes (Willson et al., 2001) (Figure 1). The downstream regulatory effects of PPAR-γ are extremely essential. This is evident in that the complete deletion of genes coding for PPAR-γ proteins (i.e., when all PPAR-γ proteins are nonfunctional in all body cells) has been shown to be lethal in embryos (Kulkarni et al., 2012).
PPAR-γ expressed in vascular-associated cells appears to have a multitude of regulatory functions in the cardiovascular system. Ligand activation in endothelial cells, vascular smooth muscle cells, and in macrophages has been shown to have positive effects on the vasculature (Duan et al., 2008). This includes a decrease in cell migration, in inflammation, in dysfunction, in ox-LDL build up, in attachment of monocytes to the endothelium, and in an increase of apoptosis in vascular smooth muscle cells, therefore, decreasing the incidence of atherosclerosis formation. This indicates a strong correlation between PPAR-γ activation and the maintenance of cardiovascular health.

Another critical discovery regarding PPAR-γ was that the aforementioned TZDs are strong agonists of PPAR-γ. TZDs are insulin-sensitizing drugs that have been used to treat type-II diabetic patients (Lehmann et al., 1995). PPAR-γ activation from TZDs has been shown to increase not only insulin sensitivity, but also the baroreflex gain in a rat model (Zhao et al., 2012), adding to the evidence that PPAR-γ is a potential mediator in cardiovascular disease.

Cardiovascular diseases and PPAR-γ function appear to be linked. A lot of investigation has been undertaken in order to determine roles of PPAR-γ receptor protein in cardiovascular diseases such as hypertension, atherosclerosis, and thrombosis, to name a few. PPAR-γ’s expression in vascular associated cells (endothelial, smooth muscle, and macrophages) is a possible link to preventing vascular dysfunction. This paper aims to examine the evidence for this link, as well as address whether PPAR-γ expressed in vasculature-associated cells has a protective role against vascular dysfunction.
Figure 1: A simplified diagram of the RXR/PPAR-γ heterodimer in cytosol and the nucleus of a cell. 1. The PPAR-γ ligand binds, causing co-repressors to leave and co-activators to bind. 2. PPAR-γ/ligand/co-activator complex moves into the nucleus and RXR binds to PPAR-γ. 3. RXR/PPAR-γ then binds to target genes and transcription starts (Kelly, 2001).
**Specific Question:**

Does PPAR-γ expressed in vasculature-associated cells have a protective role against vascular dysfunction?

**Narrow Review:**

One course of action for investigating the role of a protein in the body is to evaluate the effects of a mutation in the gene of interest. In a 1999 study conducted on humans, the authors found that PPAR-γ mutations were present in a host of subjects suffering from type-II diabetes mellitus, insulin sensitivity, and hypertension (Barroso et al., 1999). From their findings, the authors hypothesized that PPAR-γ’s expression in endothelial cells and vascular smooth muscles cells could be the possible reason for constant hypertension in the study’s participants with deleterious mutations in the PPAR-γ gene.

Through mutation studies, PPAR-γ has been found to play a key role in preventing endothelial dysfunction (Beyer et al., 2008a). One mouse model was bred to replicate the mutation found in the patients from the previously noted study by Barroso et al. (1999). This mutation of the PPAR-γ gene results in the replacement of the proline at position 465 in the protein amino acid sequence with a leucine (abbreviated as P465L). It was shown in the mouse model to induce hypertension at the early stages of mouse development (Tsai et al., 2004) (Figure 2). This result seems to be independent of the development of insulin resistance that is commonly seen in patients and animal models with PPAR-γ mutations.

**Figure 2 Analysis:**

**Context:** This study (Tsai et al., 2004) relates to this paper because the authors were intent on adding further evidence to the role of PPAR-γ in cardiovascular health. The authors used one of
the primary techniques in defining the role of a protein: mutation. In this study they found that a specific PPAR-γ mutation results in vascular dysfunction.

**Methods:** Mice were bred to have the missense mutation P465L in the PPAR-γ gene using PCR-based site-directed mutagenesis. Mutant mice and control mice were monitored for systolic and diastolic blood pressure using telemetry for a fourteen day period, as well as with the use of a tail-cuff for a 6 day period. The tail cuff method was used on 14 to 16-week old male and female mice. Telemetry was used in 24-week old female mice.

**Results:** Figure 2A: 16-week-old male wild type mice had lower BP (111 mm Hg) than PPAR-γ mutant males (120 mm Hg) using the tail cuff method (P < 0.01). Female wild type mice also had significantly lower BP (115 mm Hg) than the mutant counterparts (124 mm Hg) using the tail cuff method after 16 weeks (P < 0.01). Figure 2B: Use of telemetry to measure systolic and diastolic blood pressure in 24-week old female mice found that control mice had significantly lower BP (110/82 mm Hg) than the experimental group (132/94 mm Hg; P < 0.01) during the light cycle. During the dark cycle, results were similar with control mice BP (124/95 mm Hg) being significantly lower than the test group’s BP (144/103 mm Hg; P < 0.01).

**Relevance:** These results and this figure are relevant because they show direct negative physiological effects on the cardiovascular system from a mutation in the PPAR-γ protein.
Figure 2: Elevated BP in PPAR-γ<sup>P465L/+</sup> mice. (A) BP of 14- to 16-week-old wild-type (white bars) and PPAR-γ<sup>P465L/+</sup> (black bars) mice by tail-cuff measurement. Numbers of mice are inside bars. P < 0.01 for genotype effect by ANOVA for both sexes. (B) Four-day telemetric recordings of systolic and diastolic BP in 24-week-old female mice. Results are expressed as mean of four wild-type (dashed lines) and four PPAR-γ<sup>P465L/+</sup> (solid lines) mice averaged with 12 values each hour. Bolded bars on the x axis represent the dark cycles (Tsai et al., 2004).
Further evidence shows that mutation of the PPAR-γ protein in specific cells causes vascular dysfunction and hypertension. PPAR-γ present in vascular smooth muscle cells appears to regulate vascular tone via mechanistic interactions with vascular endothelial cells (Halabi et al., 2008). Mutation of the PPAR-γ gene specific to vascular smooth muscle cells has been found to have an array of negative cardiovascular effects in mice. Aortic dilation is impaired, hypertension is observed, and response to the vasodilator nitric oxide is diminished (Halabi et al., 2008). This evidence points to a cardio-protective role of PPAR-γ expressed in vascular smooth muscle cells.

Another mechanism that the PPAR-γ protein seems to be involved in is the baroreflex. The baroreflex is a primary physiological response to sudden changes in blood pressure, adjusting heart rate and arteriole diameter to maintain homeostasis (Van de Vooren et al., 2006). The baroreflex has been found to be significantly impaired in rats with PPAR-γ gene mutations in structural cells of the vasculature (McCully et al., 2011). This was hypothesized to be due to the expression of PPAR-γ protein in endothelial cells and vascular smooth muscle cells, both associated with the structure of the baroreceptors.

Although it is apparent that PPAR-γ gene mutations cause vascular dysfunction, the mechanism is still unclear. This is evident in that PPAR-γ proteins support resistance vessels more than larger vessels, such as the aorta versus the cerebral arterioles (Beyer et al., 2008a). For example, in the cerebral arterioles of mice, PPAR-γ gene mutation was associated with notably more dysfunction than was observed with a mutation in the aorta (Beyer et al., 2008a). The cerebral vessels responded more to vasoconstrictors and less to vasodilators when the PPAR-γ gene was mutated, decreasing overall cerebral circulation due to decreased diameter (Figure 3A), increased wall thickness (Figure 3B), increased vessel cross-sectional area (see
Figure 3C), and increased mean blood pressure (see Figure 3D). One possible explanation of this was the increase of superoxides observed in the resistance vessels that was not seen in the aorta (Beyer et al., 2008a). This suggests that one mechanism of the PPAR-γ protein’s protective role is decreasing the amount of superoxides expressed in the circulatory system.

**Figure 3 Analysis:**

**Context:** This study (Beyer et al., 2008a) relates to the specific question because the authors use genetic techniques in order to better understand the role of PPAR-γ proteins in the maintenance of vascular health. Specifically the authors reported in this figure the physical changes induced in cerebral arterioles in mice with PPAR-γ gene mutations.

**Methods:** 15 mice with the heterozygous mutation P465L in the PPAR-γ gene, denoted as L/+, and 19 control mice, denoted as +/+, were examined at 5 to 9 months of age. To obtain cerebral arteriole diameter, thickness, and cross-sectional area (CSA), mice were euthanized and cerebral arterioles were removed for measurements. Mean arteriole pressure was measured in anesthetized mice.

**Results: Figure 3A:** External diameter of cerebral arterioles in L/+ mice after maximum dilation was significantly smaller (54 mm) than external diameter in +/+ mice (64 mm; P < 0.05). Internal diameter in L/+ mice was also significantly smaller (48 mm) than internal diameter of +/+ mice (59 mm; P < 0.05). **Figure 3B:** Wall thickness in L/+ mice was significantly thicker (2.7 µm) than the +/+ mice (1.9 µm; P < 0.05). **Figure 3C:** Cross-sectional area was significantly larger (460 µm²) in L/+ mice than in +/+ mice (375 µm²; P < 0.05). **Figure 3D:** Relationship between mean arteriole pressure (mm Hg) and arteriole diameter (µm) in mutant and control mice. As mean arteriole pressure increased, so did arteriole diameter for both L/+ and +/+ mice.
Figure 3: Structural and Mechanical Changes in Cerebral Arterioles in P465L mice.
External and internal diameter of cerebral arterioles after maximal dilation (A), wall thickness (B), cross-sectional area (CSA) of the vessel wall in arterioles (C), pressure-internal diameter relationships in arterioles during maximal dilation (D), in wild-type (+/+) and L/+ mice.
*, P < 0.05 (Beyer et al., 2008a).
**Relevance:** This data is relevant because it shows direct evidence that PPAR-γ in vascular-associated cells is important for maintaining vascular health. The decrease in diameter during maximum dilation for L/+ mice (A), the increase of arteriole wall thickness in L/+ mice (B), and the increase in CSA in L/+ mice (C) all imply that PPAR-γ is relevant in preventing predisposition to hypertension and hypertrophy in at least the cerebral arterioles.

Another mechanism responsible for further understanding of how the PPAR-γ protein is involved in vascular function is through the use of thiazolidinediones (TZDs). TZDs are insulin sensitizing drugs used to treat type-II diabetic patients and are known to be agonists of PPAR-γ proteins (Zhao *et al*., 2012). Several studies indicate that TZDs activate PPAR-γ proteins, inducing antihypertensive effects and decreasing blood pressure under high-fat conditions (Zhao *et al*., 2012; Nicol *et al*., 2005). One example of this has been found with the baroreflex.

The baroreflex has been found to be diminished under high-fat diet conditions (Zhao *et al*., 2012). When TZDs were administered to obese rats by Zhao *et al.* in 2012, it was found that not only was insulin sensitivity improved, but spontaneous baroreflex gain was as well (Figure 4). The authors found the mechanism for this response to be in the activation of PPAR-γ proteins associated directly with the baroreceptor cells in the aorta and carotid sinuses (Zhao *et al*., 2012).

**Figure 4 Analysis:**

**Context:** This particular study (Zhao *et al*., 2012) relates to this research analysis because the authors investigated how PPAR-γ protein is involved in a specific vascular-associated function: the baroreflex. Using TZDs, they evaluated the effects these PPAR-γ agonists had on spontaneous baroreflex gain in mice on a high-fat diet.
Figure 4: Baroreflex gain in obesity prone (OP), obesity resistant (OR), and control (CON) rats treated with vehicle, 3 mg/kg, or 6 mg/kg rosiglitazone. Two-way ANOVA revealed significant group (P < 0.05) and rosiglitazone treatment (P < 0.0001) effects, as well as a group by treatment interaction (P < 0.05). *, P < 0.05 compared with OP, within treatment. †, P < 0.05 compared with vehicle, within group. Numbers in bars indicate number of rats (Zhao et al., 2012).
Methods: Sprague-Dawley rats were fed a high-fat diet in order to induce obesity. The high fat diet consisted of 33% kcal as fat while the control diet consisted of 13% kcal as fat. After 2 weeks on the diets, rats were observed to split into 2 groups: obesity prone (OP) and obesity resistant (OR). These distinctions were based on relative body weight gain. OP rats gained significant weight (top tertile) while OR rats did not (bottom tertile). Rosiglitazone was then administered for 2 to 3 weeks at a dose of 3 mg/kg per day or a dose of 6 mg/kg per day. Control rats (CON) were given a daily vehicle treatment. Spontaneous baroreflex gain was measured using arterial catheters while administering vasoconstrictors and dilators. Blood pressure and heart rate were recorded and used to calculate spontaneous baroreflex gain (bpm/mmHg), a measurement of baroreflex function.

Results: OP rats had a significantly improved baroreflex gain in dose-dependent manner. The OP rats treated with 3 mg/kg of rosiglitazone had significantly higher baroreflex gain (3.8 bpm/mm Hg) than rats treated with vehicle (2.9 bpm/mm Hg; P < 0.05). OP rats treated with 6 mg/kg of rosiglitazone had a significantly higher baroreflex gain (5.2 bpm/mm Hg) than OP rats given the vehicle (2.9 bpm/mm Hg; P < 0.05). OR and CON rats treated with vehicle had significantly higher baroreflex gains (3.9 bpm/mm Hg for both) than OP rats in the same treatment group (2.9 bpm/mm Hg; P < 0.05). OR rats treated with 6 mg/kg of rosiglitazone had a significantly higher baroreflex gain (5.5 bpm/mm Hg) than OR rats treated with the vehicle (3.9 bpm/mm Hg; P < 0.05).

Relevance: This figure shows the effect of a specific PPAR-γ agonist on the baroreflex, a mechanism of the cardiovascular system. This indicates that PPAR-γ could be important for function of the baroreflex and is possibly inhibited by high-fat conditions.
Although TZDs have been important in discovering the role of PPAR-γ proteins in the cardiovascular system, this drug family may actually be responsible for fatal cardiovascular events. In 2007, a study by Nissen and Wolski found that the use of rosiglitazone caused a significant risk of myocardial infarction in treated diabetic patients. However, this finding is disputed on the basis that the meta-analysis may have had many flaws and was controversial (Diamond et al., 2007), leading some authors to conclude that the risk of myocardial infarction after rosiglitazone treatment may still be present, but much lower than previously thought.

Therefore, TZDs currently remain one of the choice vehicles for studying the protective effects of PPAR-γ despite possible dangerous cardiac effects.

So far evidence has been shown for the PPAR-γ protein’s involvement in hypertension, vascular tone dysfunction, and the baroreflex. PPAR-γ is also important for further understanding of the pathogenesis and possible treatment of atherosclerosis. Important in the progression of atherosclerosis is the proliferation, recruitment, and migration of vascular smooth muscle cells (Duan et al., 2008). Inflammation and damaged endothelial cells are also crucial components in the development of atherosclerosis (Duan et al., 2008; Davis et al., 2003). The damaged endothelial cells recruit vascular smooth muscle cells to the site of injury, leading to the progression of atherosclerosis. This migration of vascular smooth muscle cells appears to be hindered by PPAR-γ activation via TZDs such as pioglitazone (Duan et al., 2008), supporting PPAR-γ’s protective role in atherogenesis.

However, it is possible that PPAR-γ could have negative effects on cardiovascular health through pro-atherogenic pathways. One example of this was found by Jung et al. in 2012 in the study of atherosclerosis-associated macrophages. Since macrophages are main components of atherogenesis, understanding macrophages has large implications. As it turns out, macrophages
associated with atherosclerotic plaques express PPAR-γ at high levels (Jung et al., 2012). This expression of PPAR-γ, however, is not fully understood. Jung et al. found that with activation of PPAR-γ protein using rosiglitazone, there was increased expression of endothelial lipase, an enzyme highly expressed in atherosclerosis. Endothelial lipase is produced by endothelial cells and vascular smooth muscle cells. The primary function of this enzyme is high-density lipoprotein (HDL) metabolism (Choi et al., 2002).

Furthermore, Jung et al. (2012) found that when GW9662, an antagonist of PPAR-γ, was used, there was a decrease in endothelial lipase expression. Along with this finding, the authors found that a specific saturated fatty acid, palmitic acid (PA), was responsible for increasing PPAR-γ mRNA expression (Figure 5). Therefore, if this specific fatty acid is increasing PPAR-γ expression and PPAR-γ expression is raising the levels of endothelial lipase, then PPAR-γ may be involved in the progression of atherosclerosis. Yet, even with these findings, Jung et al. brought up discussion points that lean towards PPAR-γ proteins possibly having an anti-inflammatory role by decreasing the expression of other inflammatory cytokines, such as TNF-α, despite increasing the levels of endothelial lipase.

**Figure 5 Analysis:**

**Context:** This paper (Jung et al., 2012) is relevant because it takes into consideration the negative effects PPAR-γ possibly has on vascular health. In this study, the authors specifically looked at macrophages associated with atherosclerosis and their expression of PPAR-γ mRNA and how up-regulation of PPAR-γ mRNA affected the progression of atherosclerosis.
Figure 5: Effects of fatty acids (FA) on macrophage peroxisome proliferator activated receptor gamma (PPAR-γ) mRNA expression. J774 (mouse-like macrophages) (A) or peritoneal macrophages from mice (B) were incubated with 150 µmol/L of eicosapentaenoic acid (EPA) or palmitic acid (PA) as previously described. Means with unlike letters are significantly different at P < 0.05 (1-way ANOVA). LPS indicates lipopolysaccharide; BSA, bovine serum albumin (Jung et al., 2012).
**Methods:** Mouse-like macrophages (cell line denoted as “J774”) and peritoneal macrophages from mice were cultured in order to observe results across two macrophage cell lines. Cells were either given bovine serum albumin (control), 150 µmol/L of eicosapentaenoic acid (EPA), or 150 µmol/L of palmitic acid (PA). The groups were also given 1 µg/mL of lipopolysaccharide (LPS) or no LPS, because LPS is a known endotoxin that increases the expression of endothelial lipase.

**Results:** Figure 5A: In the J774 cells, PA, in the presence of LPS (LPS+) and not in the presence of LPS (LPS−), elicited a significantly higher expression of PPAR-γ mRNA (4.1 and 3.6 arbitrary units, respectively) than the LPS− control (1 arbitrary unit), the LPS+ control (1.5 arbitrary units) and both EPA groups (LPS−:1.4, LPS+: 1.3 arbitrary units; P < 0.05). Figure 5B: In peritoneal macrophages treated with PA, LPS− cells had significantly higher levels of PPAR-γ mRNA expression (2.3 arbitrary units) than LPS− controls (1 arbitrary unit). LPS+ cells also had significantly higher PPAR-γ mRNA expression (2.8 arbitrary units) than LPS+ control cells (1.8 arbitrary units; P < 0.05). LPS+ control peritoneal macrophages had a significantly higher expression of PPAR-γ mRNA (1.9 arbitrary units) than LPS+ EPA treated macrophages (1 arbitrary unit; P < 0.05).

**Relevance:** This figure is relevant because it demonstrates that ligands that are considered as unhealthy, such as the specific fatty acid PA, provoked an increased expression of PPAR-γ mRNA. This figure sets the foundation for linking PPAR-γ mRNA expression and atherogenesis.

In summary, PPAR-γ proteins have been shown to support cardiovascular health and to possibly have negative effects. Mutation of the PPAR-γ gene was correlated with vascular dysfunction, inflammation, hypertension, hypertrophy, baroreflex dysfunction, and an increase in superoxides (Barroso et al., 1999; Beyer et al., 2008a; Beyer et al., 2008b; Duan et al., 2008;
Halabi et al., 2008; Hamblin et al., 2010; McCully et al., 2011). Activation of PPAR-γ proteins through agonists revealed decrease in inflammation, in superoxides, and in hypertension, and revealed an increase in baroreceptor and vascular function (Jung et al., 2012; Lehmann et al., 1995; Ryan et al., 2004; Zhao et al., 2012). Activation also showed that the formation of atherosclerotic plaques could be hindered. However, it was here that some researchers also discovered that PPAR-γ mRNA expression in macrophages may increase the pathogenesis of atherosclerosis (Jung et al., 2012).

**Conclusion:**

As can be seen from the extensive PPAR-γ protein literature, PPAR-γ proteins are important players in cardiovascular health and they do predominately have a protective role against vascular dysfunction. However, they may also be negatively associated with some cardiovascular diseases. What is clear is that mutation of the genes coding for PPAR-γ causes vascular dysfunction, and, therefore, implies that PPAR-γ is important for maintaining normal vascular health. What is also notable is that the PPAR-γ protein has the potential for being negatively involved in cardiovascular diseases such as atherosclerosis and myocardial infarction. What is unclear is whether activation from synthetic or natural ligands is the cause of such diseases.

Further investigation is needed in order to continue the process of discovering the full set of functions of PPAR-γ proteins and possibly applying that knowledge to the clinical setting. Questioning whether PPAR-γ protein expression is directly related to myocardial infarction and macrophage-associated atherosclerosis, or if these seemingly negative effects are caused by other mechanisms, is the next step to using PPAR-γ as a therapeutic mediator in cardiovascular disease.
**Broader Implications:**

As cardiovascular disease continues to rise and be a major killer in the United States, it is important to find ways to combat it. If this can be done through drug treatment that involves PPAR-γ proteins, then it could be possible to lower the incidence of cardiovascular diseases in the U.S. However, as it has been shown that PPAR-γ proteins potentially have negative effects on vascular health, simply medicating cardiovascular disease through PPAR-γ may just mask the underlying problem, delay inevitable diseases, or even cause further problems (as seen in the atherosclerosis/macrophage study by Jung *et al.*, 2012). A better step would be to take preventative measures against the causes of cardiovascular disease, such as diet-induced obesity. Removing the cause could potentially be more effective than simply treating with drugs. For those who have vascular dysfunction due to PPAR-γ gene mutations, further research is needed in order to find ways to counteract the effects of the mutation, such as gene therapy, protein replacement therapy, or drugs that mediate the effects. Overall, PPAR-γ proteins in vascular-associated cells have been and, for the time being, will continue to be of interest in understanding and treating cardiovascular disease. Understanding what in our environment, diet, and body processes affect PPAR-γ proteins will be important in future research for public awareness and potential treatment of cardiovascular diseases.
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Literature Cited


