REVIEW

The Role of PPARγ in Cardiovascular Diseases

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Summary

The peroxisome proliferator-activated receptors (PPAR) belong to the nuclear superfamily of ligand-activated transcription factors. PPARγ acts as a nutrient sensor that regulates several homeostatic functions. Its disruption can lead to vascular pathologies, disorders of fatty acid/lipid metabolism and insulin resistance. PPARγ can modulate several signaling pathways connected with blood pressure regulation. Firstly, it affects the insulin signaling pathway and endothelial dysfunction by modulation of expression and/or phosphorylation of signaling molecules through the PI3K/Akt/eNOS or MAPK/ET-1 pathways. Secondly, it can modulate gene expression of the renin-angiotensin system – cascade proteins, which potentially slow down the progression of atherosclerosis and hypertension. Thirdly, it can modulate oxidative stress response either directly through PPAR or indirectly through Nrf2 activation. In this context, activation and functioning of PPARγ is very important in the regulation of several disorders such as diabetes mellitus, hypertension and/or metabolic syndrome.

Key words

PPARγ • Hypertension • Oxidative stress • Antioxidant response • Renin-angiotensin system • Nitric oxide

Introduction

Peroxisome proliferator-activated receptors (PPAR) belong to the nuclear superfamily of ligand-activated transcription factors. The name of this group of nuclear receptors was derived from their ability to stimulate proliferation of peroxisomes in rats (Issermann and Green 1990). In humans, three isoforms of nuclear receptors were discovered: PPARα, PPARβ/δ and PPARγ, which differ in their distribution and specific function (Leonardini et al. 2009). PPAR activation leads to transactivation and transrepression of selected gene expression. Ligand-activated PPAR creates heterodimer with the retinoic X receptor, which is subsequently bound on sequence-specific target element in promoter region of target genes (Oyekan 2011).

PPARs are nutrient sensors that regulate a number of homeostatic functions, such as glucose and lipid metabolism by uptake, utilization, oxidation and storage of fatty acids. It was found that PPARγ as a nutrient-sensing signaling sensor can influence nutritional programming of hypertension and metabolic syndrome (Tain et al. 2016). PPARs participate in the regulation of cell growth, migration and apoptosis and they may modulate oxidative stress, antioxidant response and inflammatory diseases in cardiovascular system (Chen et al. 2008, Oyekan 2011, Polvani et al. 2012). Disruptions of these pathways can result in the development of several diseases and pathological states, such as obesity, obesity-induced inflammation, atherosclerosis, diabetes mellitus, metabolic syndrome and hypertension (Duan et al. 2009, Oyekan 2011, Kiss et al. 2013, Montanez et al. 2013).

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Post-translational modifications of PPARγ

Up to this day, seven diverse mRNA transcripts of PPARγ (γ1-γ7) were found, which are generated with different initiation types and alternative splicing of exons – five exons in terminal region (Chen et al. 2006, Christodoulides and Vidal-Puig 2010) and six exons in the open reading frame. Only PPARγ1 is expressed in heart and skeletal muscle, vascular smooth muscle cells and endothelial cells (Das and Chakrabarti 2006, Chen et al. 2006, Li and Wang 2007), while isoforms PPARγ2 or PPARγ4 are primary delimited in adipose tissue or freely presented in macrophages, colon and in adipocytes (Chen et al. 2006).

The action of PPARγ is regulated by post-translation modifications such as phosphorylation, acetylation, SUMOylation and/or ubiquitination that represent a potentially distinct feature of PPARγ and could be exploited for tissue-specific distribution and function (van Beekum et al. 2009). Phosphorylation by MAPK inhibits ligand binding (Hu et al. 1996, Zhang et al. 1996, Camp and Tafuri 1997) or increases the activity of PPARγ by the cyclin-dependent kinases (Compe et al. 2005, Iankova et al. 2006).

Likewise, PPARγ activity regulation is controlled by covalent attachment of small ubiquitin-like modifier (SUMO). SUMOylation is one of the post-translational modification responsible for the regulation of stability, nuclear-cytosolic distribution and activity of transcription factors at different Lys residues (Pascual et al. 2005, Shimizu et al. 2006, Kim et al. 2013).

Ubiquitination is yet another level of control over PPARγ activity. In the nucleus of adipocytes, the PPARγ2 protein level is decreased by the action of thiazolidinediones (TZDs). The degradation occurs in an ubiquitin-dependent manner in the AF-2 domain of PPARγ. However, the AF-1 domains of PPARγ1 and PPARγ2 are degraded by the REGγ proteasome, the type of proteasome that degrades the target substrate in an ubiquitin- and ATP-independent fashion. The PPARγ protein (with a short half-life time of ~2 hours) is polyubiquitinated and subsequent degraded by the proteasome (Hauser et al. 2000).

PPARγ-deficient and transgenic mice

Human genetic studies have revealed that PPARγ mutation in humans can result in either a gain or a loss of function. Pro12-Ala mutation can result in a loss of function mutation and has been reported to be associated with increased protection against insulin resistance and diabetes type 2 (Mori et al. 2001, Lindi et al. 2002) and a decreased incidence of myocardial infarction and/or lower diastolic blood pressure (BP) (Ostgren et al. 2003). On other hand, several other mutations such as Pro338Leu, Val338Met, Phe388Leu and Arg425Cys are all involved in loss of function mutations and have been associated with partial lipodystrophy, insulin resistance, diabetes and hypertension (Barroso et al. 1999, Agarwal and Garg 2002, Hegele et al. 2002). The ligand binding domain mutants of PPARγ with dominant negative actions stimulate associations with the nuclear receptor coexpressor (N-CoR) and the silencing mediator of retinoid and thyroid receptors (SMRT) and they inhibit activities of all three PPAR isoforms (Semple et al. 2005).

It was shown that PPARγ is required for placental, cardiac and adipose tissue development. PPARγ null mouse surviving to term following placental reconstitution exhibited a phenotypic array that included lipodystrophy, fatty liver and hemorrhages, with fatal consequences during the first week of life (Barak et al. 1999). Increased cardiac hypertrophy and/or blood pressure decrease were observed in cardiac knockout mice or generalized knockout mice (Duan et al. 2005, Duan et al. 2007)

PPARγ and mechanisms of blood pressure regulation in hypertension and metabolic syndrome

Activation of PPARγ plays an essential role in the regulation of eNOS expression in endothelial cells and increases bioavailability of the nitric oxide (NO) (Nagao and Yamaguchi 2012). Vascular PPARγ is known also as peripheral regulator of the cardiovascular rhythm. PPARγ controls circadian variation in blood pressure and heart rate via the BMAL-1 (Brain and muscle Arnt-like protein-1) protein (Wang et al. 2008) and is an important component of the “vascular clock”. Pioglitazone (PIO), the PPARγ agonist, modifies BP circadian rhythm in patients with diabetes mellitus type 2 from non-dippers (patients without 10 % BP decrease at night) to dippers (patients with 10 % or higher BP decrease at night) (Anan et al. 2007). PPARγ can play an important role in the regulation of vascular homeostasis. Disruption of PPARγ function in these processes can contribute to vascular pathologies, such as atherosclerosis, restenosis and...
hypothesis that TZDs can decrease BP in nondiabetic hypertensive patients (Raji et al. 2003) as well as in diabetic patients (Shargorodsky et al. 2003). Moreover, TZDs improve endothelial dysfunction in rat mesenteric resistance arteries after angiotensin II (Ang II) infusions (Diep et al. 2002). The beneficial pleiotropic action of PPARγ on the vasculature are glycemia-independent (Sugawara et al. 2010). This effect can occur via several mechanisms comprising PPARγ-mediated modulation of expression and/or phosphorylation of specific signaling molecules of the insulin signaling pathway. This PPARγ-dependent modulation of insulin receptor signaling can lead to re-establishment of the balance between PI3K/Akt/eNOS and MAPK/ET-1, with a subsequent increase of NO bioavailability and vasodilatation (Li and Wang 2007). Furthermore, PPARγ can directly modulate gene expression of antioxidant and prooxidant genes and maintain redox homeostasis via interaction between PPARγ and the signaling pathways involved in oxidative stress modulation. It is an example of cooperation of signaling pathways, such as Nrf2 (Nuclear factor E2-related factor 2), NF-κB (nuclear factor kappa B), Wnt (Wingless-type MMTV integration site)/β-catenin and FOXO (forkhead box protein O) proteins (Polvani et al. 2012). PPARγ activation can decrease oxidative stress in rostral ventrolateral medulla, which plays a critical role in pathogenesis of hypertension (Chan et al. 2010).

Modulation of oxidative stress can slow down the development of hypertension in the prehypertensive period (Dovinova et al. 2013).

In addition, PPARγ can inhibit gene expression of the AT1 receptor and, through Ang II, induce ERK 1/2 activation and subsequent inhibition of vascular remodeling (Benkirane et al. 2006). PPARγ activation can inhibit the renin-angiotensin system (RAS) and decrease BP (Roszer and Ricote 2010).

**PPARγ and the PI3K/Akt/eNOS signaling pathway**

Insulin plays an essential role in the control of glucose homeostasis in skeletal muscles, adipose tissue and liver. It is involved in blood flow regulation and maintenance of vascular health. The most important effect of insulin on the cardiovascular system is the stimulation of NO production in the vascular endothelium. Abnormal reduction of insulin sensitivity affects vascular homeostasis, with manifestations such as impaired endothelial function, vasodilation, enhanced vascular inflammation and atherosclerotic lesion formation.

Insulin is synthesized in pancreatic β-cells in the islets of Langerhans in response to increased glycemia after a meal from a precursor – preproinsulin (Sharma et al. 2008). Preproinsulin, 110-amino acids long, contains an N-terminal signal peptide that facilitates transfer of signal recognition particles into the lumen of the rough endoplasmic reticulum. The signal peptide of preproinsulin is subsequently cleaved away by a signal peptidase into proinsulin. Proinsulin folds in the oxidizing environment and the folded proinsulin is transported to the Golgi apparatus in immature secretory vesicles where is cleaved down to insulin and C-peptide (Fu et al. 2013). The insulin signaling pathway is activated through insulin binding to the insulin receptor (IR). The binding of insulin leads to conformation changes in the intrinsic tyrosine kinase of the insulin receptor as well as the tyrosine phosphorylation of multiple docking proteins, including insulin receptor substrates 1 (IRS1) and 2 (IRS2) (Fig. 1a). IRS1 is necessary for insulin-stimulated NO production in the endothelium. IRS1 and IRS2 are not functionally redundant, although they both activate many similar downstream pathways (Waters and Pessin 1996, Hanke and Mann 2009). IRS2 may contribute to NO production, but is primarily implicated in the delivery of insulin to skeletal muscle interstitium (Arce-Esquivel et al. 2013).

IRS2 is a widely expressed protein that regulates crucial biological processes including glucose metabolism, protein synthesis and cell survival. IRS2 is a part of the insulin signaling pathway and mediates the activation of the NO or ET-1 through PI3K/Akt or Ras/MAPK in insulin target tissues and in the pancreas (Oliveira et al. 2014). The role of the muscle vasculature is beginning to be appreciated as a factor that influences the myocyte response to insulin. Insulin signaling plays a critical role in glucose disposal in skeletal muscle (DeFronzo et al. 1985). In cultured myocytes, insulin
activates insulin receptor and IRS2 proteins and stimulates glucose uptake in within minutes (Sarabia et al. 1990, Sarabia et al. 1992, Ogihara et al. 1997). Loss of skeletal muscle capillary density is observed in both insulin resistance and in type 2 diabetes, while insulin action is positively correlated with capillary density (Lillioja et al. 1987). Insulin-stimulated increases in blood flow or increased recruitment of perfused capillaries are both dependent on nitric oxide (NO) production and may augment insulin delivery to the interstitium because NO has been shown to mediate insulin-stimulated capillary recruitment (Kubota et al. 2011).

IRS1 or IRS2 phosphorylation leads to the activation of two signaling pathways: PI3K/Akt/eNOS and MAPK/ET-1 (Fig. 1a). The tyrosine phosphorylated IRS1 activates phosphatidylinositol 3-kinase (PI3K) through phosphorylation of the src homology 2 (SH2) domain of the p85 regulatory subunit of PI3K. Activation of the p110 subunit of PI3K cleaves catalytically the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 is needed for PIP3-dependent phosphorylation of PDK1 (3'-phosphoinositide-dependent kinase-1) and Akt kinase. Finally, Akt kinase phosphorylates several effector proteins, including GSK-3β, FOXO and eNOS. GSK-3β activation is insulin-independent, but it may be activated through the insulin signaling pathway. GSK-3β phosphorylation through IRS1 activation and PKB inhibition is more present in basal conditions compared to insulin receptor stimulation (Copps and White 2012).

Fig. 1. Activation of the insulin signaling pathway and the effect of TZD-activated PPARγ. a) Activation of the insulin signaling pathway and the effect of insulin on vascular tone. b) Activation of PPARγ by TZDs. PPARγ activation affects the insulin signaling pathway by modulation of expression and/or phosphorylation of specific signaling molecules, which can lead to reestablishment of balance between the vasodilator and vasoconstrictor effects due to insulin receptor activation resulting in an improvement of insulin sensitivity.

PI3K/Akt/eNOS signaling pathway is critical point for vascular tone regulation, because eNOS catalyzes the conversion of L-arginine to L-citrulline and produces NO. On the other hand, MAPK cascade activation plays an important role in insulin-regulated mitogen processes, such as cell proliferation, cell differentiation and cell survival (Kim and Choi 2010).

The balance between vasodilator and vasoconstrictor effects induced by the activation of insulin receptor is disrupted in vascular endothelium cells of insulin resistance animal models. PI3K/Akt/eNOS signaling pathway is suppressed while signaling pathway MAPK/ET-1 is preserved (Kobayashi et al. 2004). This balance disruption is called endothelial dysfunction (Mitchell et al. 2008). TZDs activation of PPARγ improves insulin sensitivity in diabetic patients, leading to the reduction of plasma insulin and glucose levels (Fig 1b). PPARγ activation affects insulin signaling pathway by modulation of expression and/or phosphorylation of specific signaling molecules, which can lead to reestablishment of balance between vasodilator and vasoconstrictor effects due to insulin receptor activation.

Li et al. (2010) showed that PI3K expression and phosphorylation of PKB-eNOS in vascular tissues is decreased in the young male spontaneously hypertensive
rats (SHR). Administration of rosiglitazone (RSG) raises vascular PPARγ expression, resulting in restoration of PI3K/PKB/eNOS signaling activation, followed by the improvement of endothelial function in the young SHR due to increased NO release (Li et al. 2010).

In clinical practice, synthetic ligands are used. Synthetic PPARγ ligands are TZD derivates. They are selective agonists of nuclear receptor PPARγ and they are used for insulin sensitivity improvement in patients with diabetes mellitus type 2 (Nakamura et al. 2000, Fruchart et al. 2001, Taniguchi et al. 2001), while TZDs activate minimally PPAR-α and PPAR-β isoforms (Willson et al. 1996). TZDs treatment can influence and change the expression of signaling molecules of the insulin signaling cascade (IRS, PI3K, Akt, eNOS, 5′-AMP kinase, GLUT4, etc.) connected with improvement of insulin sensitivity and/or endothelial dysfunction.

TZDs comprise troglitazone, rosiglitazone, pioglitazone and tideglusib. Troglitazone was approved in 1997, but it was withdrawn from the market for increased risk of liver failure and extensive hepatotoxicity in 2000 (Kohlroser et al. 2000). Tideglusib is a potent, irreversible, non-ATP-competitive glycogen synthase kinase-3β (GSK-3β) inhibitor and PPARγ agonist tideglusib seems to be suitable candidate for Alzheimer disease treatment or for a treatment of stroke (Armagan et al. 2015), but its application in cardiovascular disease treatment has not been examined yet.

The effectiveness of other two TZDs pioglitazone and rosiglitazone are similar in treatment of diabetes mellitus type 2, but they differ in the treatment of cardiovascular diseases. The PROactive study points out the positive effect of PIO (reduced cardiovascular complications by 16 %) in cardiovascular disease patients (Dormandy et al. 2009), while RSG treatment was associated with increased incidence of myocardial infarction and death after a relatively short-term exposure (Nissen and Wolski 2007). The European Medicines Agency withdrew its approval of this medication in 2010 due to cardiovascular safety concerns (Krishnaswami et al. 2010).

**PPARγ in adipose tissue and insulin resistance**

PPARγ has tissue-specific effects. It is most highly expressed in adipose tissue. PPARγ upregulates genes involved in glucose uptake and also controls the expression of factors secreted by adipocytes in adipose tissue. PPARγ activation enhances the uptake and sequestration of fatty acids in adipose tissue and improves insulin resistance (Ahmadian et al. 2009). Members of the fibroblast growth factor family (FGF1 and FGF21) act locally in adipose tissue to mediate the physiological and pharmacological actions of PPARγ and to promote insulin sensitization in response to high-fat diet (Dutchak et al. 2012, Jonker et al. 2012, Sun and Scherer 2012). FGF binding to FGF receptors induces the activation of four key intracellular signaling pathways: RAS/RAF/MAPK, PI3K/Akt, STAT and PLCγ (Beenken and Mohammadi 2009).

FGF21 is a key mediator of fatty acid oxidation and lipid metabolism. Pharmacological doses of FGF21 improve glucose tolerance, lower serum free fatty acids and lead to weight loss in obese mice. However, the expected beneficial effects of endogenous FGF21 to increase glucose tolerance and reduce circulating triglycerides are absent in obesity and leads to FGF21-resistant state (Fisher et al. 2010).

In the development of cardiovascular diseases including atherosclerosis, coronary heart disease, myocardial ischemia, cardiac hypertrophy and diabetic cardiomyopathy, there is a strong evidence showing that the development is associated with an increase in serum FGF21 levels. This was regarded as a compensatory response to induced cardiac protection. Furthermore, administration of FGF21 suppressed the above cardiovascular diseases. Mechanistic studies revealed that FGF21 induced cardiac protection likely by preventing cardiac lipotoxicity and the associated oxidative stress, inflammation and apoptosis (Cheng et al. 2016).

**PPARγ and endothelial nitric oxide synthase**

The regulation of eNOS expression and its activation is influenced by various cellular events, such as transcription regulation, protein-protein interaction, phosphorylation and dephosphorylation at different amino acid sequences of eNOS regulated by various kinases and phosphatases (Govers and Rabelink 2001). eNOS has a few well-described phosphorylation/dephosphorylation sites, such as as Ser1177 and Thr495. Some other sites regulating NOS activity (Ser633, Ser114 and Ser615) have been identified, but their precise roles remain controversial (Mount et al. 2007). These phosphorylation/dephosphorylation regulatory eNOS sites can be activated by multiple protein kinases, including PKA, PKB, PKC, AMPK, ERK and phosphatases such as protein phosphatase (PP)1 and

Recent studies have suggested a potential regulatory role of PPARγ-dependent eNOS expression and NO generation in the vascular endothelium. Several studies suggest that a direct transcriptional mechanism can be involved in PPARγ-mediated release of NO in endothelial cells. The activation of PPARγ using 15d-PGJ2 or ciglitazone stimulates NO release from the endothelium (Calnek et al. 2003). Mechanisms of PPARγ activation-mediated increase in endothelial NO production are not yet understood.

Polikandriotis et al. (2005) suggested that PPARγ-dependent eNOS activation is mediated via hsp90-dependent mechanism. RSG and 15d-PGJ2 stimulated hsp90-eNOS interaction, resulting in eNOS activation (at Ser1177 phosphorylation). Conversely, ciglitazone did not have this effect, which suggests different effects of PPARγ agonists. It seems that hsp90 probably plays a key role in this context because coadministration PPARγ agonist with geldanamycin (inhibitor of hsp90) attenuates eNOS activation and NO release from endothelial cells (Polikandriotis et al. 2005).

PPARγ activation decreases expression of NADPH oxidase subunits such as Nox1, gp91phox (Nox2) and Nox4, which is accompanied by an enhanced expression of SOD (superoxide dismutase) resulting in reduction of oxidative stress in the membrane of human umbilical vein endothelial cells. The elevated vascular oxidative stress reduces endothelial bioavailability of NO through peroxynitrite generation. Hwang et al. (2005) suggest that, NO release from endothelium due to PPARγ activation could enhance NO bioavailability by reducing endothelial superoxide anion and oxidative stress.

Endothelial dysfunction can be influenced with adiponectin. This hormonal protein, secreted in adipose tissue, can improve endothelial function via PPARγ activation-mediated eNOS phosphorylation. RSG administration to the diabetic mouse leads to the enhancement of NO bioavailability and the improvement in endothelial function through adiponectin release and subsequently activation of AMPK/eNOS and cAMP/PKA signaling pathways in the aorta (Wong et al. 2011).

Rho-kinase plays an important role in endothelial dysfunction development. This serine threonine kinase inactivates eNOS and reduces NO bioavailability (Budzyn et al. 2006, Nohria et al. 2006). PPARγ activation inhibits up-regulation of protein tyrosine phosphatase-2 (SHP-2) Rho-kinase. PIO administration in Ang II-treated cultured rat aortic smooth muscle cells up-regulates SHP-2 and subsequently dephosphorylates a GTP/GDP exchange factor of Rho-kinase Vav, resulting in the inactivation of Rho-kinase (Wakino et al. 2004).

Several studies suggest that PPARγ plays a distinctive role in regulation of eNOS expression in the endothelium, resulting in enhanced generation of vascular NO. PPARγ activation mediates vascular anti-inflammatory and antioxidant response and regulates endothelial function. Therefore, it has a beneficial effect on the vascular function in patients with atherosclerosis and hypertension with or without diabetes mellitus (Polikandriotis et al. 2005, Duan et al. 2009, Yu et al. 2010). Moreover, the therapeutic opportunities of agents that activate PPARγ in preventing vascular endothelial dysfunction and cardiovascular disorders associated with endothelial dysfunction are discussed.

**PPARγ and renin-angiotensin system**

One PPARγ-dependent mechanism, which can influence hypertension development, is the attenuation of AT1 receptor gene expression (Diep et al. 2002, Benkiran et al. 2006, Sugawara et al. 2010). This gene suppression of AT1 receptor is a result of protein-protein interaction between nuclear receptor PPARγ and Sp1 protein (specificity protein 1 transcription factor). Sp1 belongs to the class of zinc-finger motifs presented in transcription factors, which can join to GC rich promotor regions. PPARγ either down-regulates the expression of AT1 receptor and/or inhibits signaling pathways, such as PI3K and MAPK, through Ang II, PI3K and MAPK, suppressing thus ultimately the activation of RAS cascade (Wu et al. 2004, Benkiran et al. 2006) and remodeling of vascular wall (Benkiran et al. 2006).

RAS is an endocrine system that plays an essential role in cardiovascular and renal physiology. RAS regulates BP and the concentration of sodium ions (Rodrigues-Ferreira and Nahmias 2015). It also influences the sympathetic nervous system (SNS) functions and regulates extracellular fluid volume (Majzunova et al. 2013). Dysregulation of RAS participates in the pathogenesis of hypertension, atherosclerosis, ischemic heart disease and heart failure. Physiologically, the effect of RAS is mediated through Ang II, which is generated by an enzyme cascade and released to systemic circulation (Crowley and Coffman 2012). When BP in renal arteries...
decreases, cells of the juxtaglomerular apparatus produce and secrete a protease renin (43 kDa). Renin is an endopeptidase and cleaves angiotensinogen to the decapeptide Ang I (angiotensin I). Elevated angiotensinogen concentration leads to supernormal Ang II production (Kobori et al. 2007). Ang II is a major stimulator of synthesis and secretion of aldosterone and activates SNS (Fleming et al. 2005). It is a mediator of oxidative stress, stimulates the production of cytokines, synthesis of adhesive molecules and stimulates vascular remodeling. Production of Ang II in the heart and in the vascular system significantly contributes to the pathogenesis of the cardiovascular diseases. Except for Ang II, Ang 1-7 (angiotensin 1-7) can be produced from Ang I through “alternative RAS axis”. This alternative Ang 1-7 production is catalyzed by angiotensin-converting enzyme 2 (ACE2) (Santos et al. 2013).

Ang II binds to AT1 or AT2 receptors. They are associated with GPCR (G protein-coupled receptors). Similarly, it is associated with GPCR Mas receptor. Ang 1-7 is bound to Mas receptor. Activation of these receptors results in the phosphorylation of different signaling pathways, which often leads to opposite cellular roles. ACE/Ang II/AT1 cascade mediates the major effects of Ang II. Activation of AT1 receptors stimulates L-type of calcium channels and through G-protein leads to activation of NADPH oxidase and to vasoconstriction response (Buchanan et al. 1995). Activation of ACE/Ang II/AT2 cascade plays a role especially in prenatal period, when this cascade activation acts counter-regulatory to AT1 receptor. Activation of AT2 receptors results in anti-proliferative effect and leads to vasodilatation (Carey et al. 2000).

An increased expression of various components of the RAS during hypertension has been described in detail by Zicha et al. (2014). Several kinases are activated through Ang II-activated AT1 receptor, e.g. MAPK, p70S3 kinase, etc. This kinase activation is associated with BP increase and with hypertension development. AT1 receptor activation stimulates NADPH oxidase in vascular wall, which is major source of reactive oxygen species (ROS) (Touyz and Schiffrin 1999).

Gene expression of RAS molecules can be modulated also by PPAR (Fig. 2). PPARγ is expressed in the juxtaglomerular apparatus cells. Endogenous and pharmacological PPARγ ligands stimulate renin gene expression (Roszer and Ricote 2010).

Ligands of PPARγ can suppress the gene expression of ACE in vascular tissues (Efrati et al. 2007, Takai et al. 2007, Song et al. 2008). In streptozotocin-induced diabetes in rats, bezafibrate and PIO can equally protect against the streptozotocin-induced up-regulation of ACE in the aortic wall. Similarly, ACE gene expression is reduced by chronic treatment with RSG in obese Zucker rats (Song et al. 2008). Clinical studies have demonstrated that telmisartan inhibits ACE and blocks AT1 receptor, resulting in the vascular protection conferred to hypertensive type 2 diabetic patients by the anti-inflammatory and antiatherogenic consequences of PPAR activation (Unger and Stoppelhaar 2007).

Two Ang II receptor blockers, telmisartan and irbesartan, act as selective PPAR modulators (Sanchez et al. 2008, Tagami et al. 2009). Partial PPAR agonist telmisartan inhibits vascular ACE activity (Takai et al. 2007), AT1 receptor expression (Gosse 2006, Imayama et al. 2006) and increases endothelial NO synthesis (Kobayashi et al. 2008), preventing oxidative stress and endothelial dysfunction more effectively than non-PPAR-agonists among Ang II receptor blockers.

PPAR blocks the action of Ang II by transcriptionally repressing AT1 receptor gene expression in VSMCs (vascular smooth muscle cells) (Takeda et al. 2000, Imayama et al. 2006, Sanchez et al. 2008, Tagami et al. 2009). In addition to its role as a regulator of vascular tone, AT1 receptor activation contributes to vascular lesions and atherogenesis by promoting VSMC proliferation (Nishijo et al. 1998, Wakino et al. 2001). Therefore, a suppressed Ang II response can potentially slow the progression of atherosclerosis.

Fig. 2. Role of PPARγ in the modulation of the RAS cascade, modified according to Roszer and Ricote (2010). Gene expression of RAS molecules is modulated by PPARγ. Although renin production is facilitated by PPARγ activation, the hypertensive effects of RAS activation are attenuated by PPARγ ligands, since these strongly reduce Ang II levels, ACE and AT1 receptor gene expression.
ACE inhibitor enalapril was tested on PPAR responses. Anthracycline therapy is limited by a cardiotoxicity that may eventually lead to chronic heart failure which is thought to be prevented by ACE inhibitors. Involvement of PPAR and changes in cellular metabolism in anthracycline cardiomyopathy has been investigated after enalaprilat administration to daunorubicin-treated Wistar rats. It was found that anthracycline administration significantly up-regulated PPARα mRNA in the heart, but did not influence the expression of cardiac PPARβ/δ. It the case of coadministration of ACE inhibitors the expression of cardiac PPARα mRNA did not change, while the expression of PPARβ/δ was increased. Altered expression of heart PPARs may suggest these nuclear receptors as a novel target in anthracycline cardiomyopathy (Cernecka et al. 2013).

Phosphorylation cascades involved in RAS and PPARγ regulation

Increased phosphorylation of signaling pathways participating on the growth and differentiation of cells is activated through Ang II. Ang II can subsequently activate PI3K and ERK 1/2 kinase and kinase cascades involved in these processes (Touyz and Schiffrin 1999).

PI3K activation via Ang II starts with phosphorylation of regulatory subunit 85α of PI3K. Subsequently, lipid phosphate SHIP2 (Src homology 2 domain containing inositol 5-phosphatase 2) is activated. PI3K generates PIP3 and subsequently SHIP2 phosphatase hydrolyzes PIP3 to primary lipids, which are needed for activation of Akt kinase (Franke et al. 1997, Klippel et al. 1997, Scheid et al. 2002). Akt kinase is involved in mechanisms of cell survival, growth and vascular remodeling. Gingras et al. (1998) and Kitamura et al. (1998) suggested that the activation of PI3K/Akt cascade plays an important role in protein synthesis involved in vascular remodeling and hypertrophy, although Akt kinase is a kinase of “cell survival”, because of its antiapoptotic effect (Downward 1998, Zhuang et al. 2011). Mechanisms of AT1 receptor-mediated PI3K-dependent activation of Akt kinase is not yet well understood, but it seems to play an important role in redox-sensitive signaling pathways and ROS-mediated c-Src activation (Thomas and Brugge 1997, Ushio-Fukai et al. 1999). c-Src is cytoplasmic tyrosine kinase, which is activated especially via superoxide anions generated by NADPH oxidase.

Another kinase cascades activated through Ang II are mitogen-activated protein kinase (MAPK) cascades which are universal signal transduction modules. MAPKs are activated through Ang II are ERK 1/2 (extracellular signal regulated kinase), JNK (c-Jun N-terminal kinase), p38, etc. (Robinson and Cobb 1997).

ERK 1/2 participates on regulation of expression of growth and differentiation factors. Mechanism of ERK activation is not yet completely explained. Activation starts through β and γ subunits of G protein and through receptor associated with tyrosine kinase Shc (Src homology domain) (Apostolidis and Weiss 1997, Berk and Corson 1997, Schieffer et al. 1997). Tyrosine kinase associated receptor stimulates joining p21ras receptor via a phosphorylation cascade. This receptor includes adaptor proteins with guanine nucleotide exchange factor. GDP/GTP exchange of p21ras protein is stimulated through Shc-Grb2-Sos (son-of-sevenless) complex. Grb2 (growth factor receptor-bound protein 2) is stimulated by autophosphorylation of tyrosine kinase receptor. Serine c-Raf kinase is linked to plasma membrane with p21ras protein. Serine c-Raf kinase phosphorylates MEK (MAPK/ERK kinase). MEK dually phosphorylates threonine and tyrosine residues in Thr-Glu-Tyr motive, resulting in ERK 1/2 activation (ERK 1: 44-kDa MAPK isoform; ERK 2: 42-kDa MAPK isoform) (Touyz and Schiffrin 2000). ERK 1/2 phosphorylation cascade plays a role in regulation of expression of growth and differentiation factors. In vitro studies suggested that PPARγ activation can inhibit activation of ERK 1/2 induced by Ang II (Goetze et al. 1999, Takeda et al. 2000, Diep et al. 2002, Fukuda et al. 2002).

Effect of PPARγ on oxidative stress and cell stress response regulated by Nrf2

Oxidative stress represents disruption of redox homeostasis for benefit of prooxidants, which exceed antioxidant capacity of cell, resulting in potential damage (Sies 2007). ROS produced in low concentration by different ROS sources in organism are an integral part of several intracellular signaling pathways during physiological processes (Touyz et al. 2003).

ROS are main contributors of oxidative stress in cells during physiological and pathological processes. The main enzyme sources of ROS include mitochondrial respiratory chain, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase and
uncoupled NO synthase. They are controlled under physiological conditions using cell stress detection of Nrf2 signaling pathway. This cellular protection system is activated in pathophysiologic condition during oxidative and electrophilic stress (Elahi et al. 2009, Rodrigo et al. 2011, Touyz and Briones 2011).

The Keap1/Nrf2/ARE is a complex system maintaining redox homeostasis in normal or stress conditions. These signaling pathways consist from Nrf2 protein/nuclear factor, Keap1 protein and ARE element (antioxidant response element) (Nguyen et al. 2009). The Keap1 protein regulates negatively Nrf2 activity through cysteine residues which are sensitive to presence of ROS (Kang et al. 2004). During oxidative stress Nrf2 can be released from connection with Keap1 and is translocated to the nucleus where induces transcription of genes by ARE (Hur et al. 2010, Tkachev et al. 2011). ARE is present within regulatory areas of several cytoprotective target genes directly involved in the production of antioxidant and detoxification proteins (Reichard et al. 2007, Gardlik et al. 2011, Majzunova et al. 2013).

Further production of detoxification and antioxidant proteins is involved in the elimination of reactive oxidants and electrophilic products. These processes are performed through conjugation reactions detoxifying of xenobiotics by production of enzymes in detoxification phase II (e.g. glutathione-S-transferase, NAD(P)H: quinone oxidoreductase 1), direct inactivation of ROS and increasing the antioxidant capacity of cells (e.g. catalase, superoxide dismutase, heme oxygenase-1) and regulation of glutathione production (e.g. glutamate-cysteine ligase) (Ishii et al. 2002, Gardlik et al. 2011, Hur and Gray 2011).

During chronic stress, some mechanisms appear to “switch off” Nrf2 activity. This process occurs after Nrf2 inactivation by aberrant phosphorylation by Fyn (fgr/yes novel protein) and GSK3β (glycogen synthase kinase 3β). GSK3β phosphorlates Fyn which affected phosphorylation of Nrf2 in nucleus. This action results in the export of Nrf2 from the nucleus to the cytosol and binding to Keap1 with the subsequent degradation of Nrf2 (Jain and Jaiswal 2006, Kanninen et al. 2011).

**Connection between PPARγ and Nfr2**

PPARγ maintains redox homeostasis by activation and repression of several signaling pathways. In the case of Nrf2 there is a reciprocal transcriptional regulation between genes of Nrf2 and PPARγ. Nrf2 contains putative PPREs and counter PPARγ gene contain ARE (Park et al. 2004, Cho et al. 2010). Experiments with Nrf2 null mice show that expression of PPARγ is reduced due to direct effect of Nrf2 lack. Nrf2 induces PPARγ expression by binding to at least two ARE sequences in the upstream promoter region of the nuclear receptor (Huang et al. 2010, Cho et al. 2010). Furthermore, Nrf2 expression is weakened in mice with decreased PPARγ (Park et al. 2004). A positive feedback loop between PPARγ and Nrf2 probably exists, where PPARγ may act directly or through upstream pathway for Nrf2 activation (Polvani et al. 2012). In addition, nuclear receptor PPARγ and transcription factor Nrf2 may act synergically in the activation of antioxidant genes (Ishii et al. 2004, Park et al. 2004). ARE and PPRE response element coexist in GSTA2 promoter. GSTA2 gene encodes detoxification enzyme glutathione S-transferase A2, where ARE site is important in the transactivation of the GSTA2 gene by PPARγ and RXR ligands for the full gene transactivation (Park et al. 2004). Similar expression of CD36 is dependent on both proteins, but transcription of CD36 may be induced independently by Nrf2 or PPARγ, depending on the cellular context (Ishii et al. 2004).

There are another ways of activation and repression of antioxidant and prooxidant pathways. PPARγ creates a heterodimer with RXR and together regulate gene transcription (Desvergne and Wahli 1999, Varga et al. 2011). As dimers, PPARγ:RXR bind to PPRE located in the promotor region of target genes. PPARγ may directly modulate the expression of several antioxidant and prooxidant genes in response to oxidative stress. It was observed that lipid-activated PPARγ promotes the expression of manganese SOD (MnSOD) (Ding et al. 2007), GPx3 (Chung et al. 2009), the scavenger receptor CD36 (Ishii et al. 2004), heme oxygenase-1 (HO-1) (Kronke et al. 2007) and the mitochondrial uncoupling protein 2 (UCP2) (Chan et al. 2010), whereas it down-regulates COX-2 and iNOS (Zhao et al. 2006, Vandewalle et al. 2008, Polvani et al. 2012).

One mechanism of PPARγ action represents the coexistence of PPREs and ARE in the same genes. The expression of scavenger receptor CD36 (mediates the recognition and internalization of oxidized lipids, antioxidant enzymes catalase, HO-1 and mitochondrial MnSOD are transcriptionally regulated through PPREs (Ishii et al. 2004, Ding et al. 2007, Kronke et al. 2007).
Redox homeostasis disruption in cardiovascular diseases and the role of PPARγ

Disruption of redox homeostasis and pathophysiological ROS level leads to series of damage effects that participate in the development of cardiovascular diseases. Hypertension is a multifactorial complex disease leading to the damage of several organ systems. The factors participating in hypertension development include SNS activation, RAS up-regulation, altered G protein-associated signalization and inflammation (Touyz 2005, Harris et al. 2008). Oxidative stress is a common factor of these processes. It affects processes participating in vascular remodeling, growth and proliferation of vascular smooth muscle cells, lipid peroxidation, extracellular matrix remodeling, fibrosis, monocyte invasion and inflammation. Moreover, increased ROS levels are associated with higher vascular contractility, vascular and cardiac fibrosis and remodeling, manifested by blood pressure elevation and cardiac output increase (Montezano and Touyz 2014).

Polvani et al. (2012) pointed out that nuclear receptor PPARγ can affect redox homeostasis by interacting with signaling pathways, including nuclear factor Nrf2, NF-κB, Wnt/β-catenin and FOXO proteins (Fig. 3).

In the pathogenesis of hypertension it was observed that ROS overproduction in the central nervous system increases SNS activation in peripheral vessels (Peters et al. 2000, Nagao and Yamaguchi 2012). Furthermore, increased AT1 receptor expression, almost localized on glutamatergic neurons in rostral ventrolateral medulla (RVLM), can modulate excitatory or inhibitory neurons and their postsynaptic activity (Hu et al. 2002). This can lead to higher SNS activity and positive correlation with BP (Dampney et al. 2007).

Moreover, ROS overproduction in the kidneys increases Ang II production and Ang II-mediated renal vasoconstriction (Just et al. 2008), renin release (Welch et al. 1983), renal nervous activity (Lai et al. 2012) as well as the activation of endothelin-1 and thromboxane (Wang et al. 2004).

TZDs treatment decreases ROS production in vascular smooth muscle cells (Dobrian et al. 2004, Ceolotto et al. 2007) and endothelial cells (Dobrian et al. 2004, Sorrentino et al. 2007), allowing the decrease in blood pressure. Chan et al. (2010) suggest that RSG administration shows central antihypertensive effect. With respect to the central role of oxidative stress in neural mechanisms of hypertension development (Peters
et al. 2000, Kimura et al. 2005), the antihypertensive effect of RSG can be mediated through PPARγ-dependent transcriotional up-regulation of antioxidant mechanisms in RVLM (Chan et al. 2010).

PPARγ protects against the apoptosis of cardiomyocytes and glial cells from oxidative stress by induction of transcription of Bcl-2 (B-Cell CLL/Lymphoma 2) (Ren et al. 2009).

In humans vessels, the expression of HO-1 is induced in the presence of oxidative stress and high glucose level after TZDs administration (Wang et al. 2011). Oxidative stress in RVLM, where sympathetic premotor neurons reside, contributes to sympatho-excitation and hypertension. Metabolic syndrome, which is activated in rats by high-fructose diet, increases sympathetic vasomotor activity, blood pressure and ROS level, while it reduces NO and mitochondrial electron transport capacity. Treatment of these animals by TZDs (PIO) abrogated all those molecular events in this model of metabolic syndrome and ameliorated sympathoexcitation and hypertension (Wu et al. 2014).

Conclusions
The PPARγ nuclear receptor is transactivated by its ligands and functions as an activator or repressor of a variety of signaling pathways. It may be also involved in insulin signaling regulation, RAS suppression, as well as in the regulation of antioxidant response, either directly or through the Nrf2 transcription factor.

PPARγ regulates a number of homeostatic functions of the glucose and lipid metabolism and, as a nutrient-sensing signaling sensor, it can influence nutritional programming to hypertension and/or metabolic syndrome. Several ligands of this receptor can potentiate its activation. Many exogenous ligands such as thiazolidinediones: rosiglitazone, pioglitazone or tideglusib have a range of positive effects on cardiovascular diseases and the metabolic syndrome, however, some of them have harmful side effects. The usefulness and effectiveness of PPARγ ligands on lifestyle-related diseases has yet to be established. Future investigations have to search for various endogenous ligands and their effects.

Conflict of Interest
There is no conflict of interest.

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Abbreviations
(PP)1 – protein phosphatase 1
15d-PGJ2 – 15-deoxy-A-12,14-prostaglandin J2
ACE – angiotensin converting enzyme
AF-1 and AF-2 – activation function motive 1 and 2
Akt – Akt serine/threonine kinase 1
AMPK – AMP-activated protein kinase
Ang 1-7 – angiotensin 1-7
Ang I and Ang II – angiotensin I and II
ARE – antioxidant response element
AT1 and AT2 – angiotensin-receptor type 1 and 2
Bel-2 – B-cell CLL/Lymphoma 2
BMAL-1 – brain and muscle Arnt-like protein-1
BP – blood pressure
cAMP – cyclic adenosine 3’, 5’ monophosphate
CD36 – cluster determinant 36
COX-2 – cyclo-oxygenase 2
c-Src – c-Src tyrosine kinase
eNOS – endothelial nitric oxide synthase
ERK 1/2 – extracellular signal regulated kinase
ET-1 – endothelin 1
FGF – fibroblast growth factor
FOXO – forkhead box protein O
Fyn – fgr/yes novel protein
GC rich promoters regions – guanine and cytosine rich promoters regions
GDP – guanosine diphosphate
GLUT4 – glucose transporter type 4
GPCR – G-protein-coupled receptors
GPx3 – glutathione peroxidase 3
Grb2 – growth factor receptor-bound protein 2
GSK-3β – glycogen synthase kinase-3beta
GSTA – glutathione S-transferase A2
GTP – guanosine-5’-triphosphate
HO-1 – heme oxygenase-1
iNOS – inducible nitric oxide synthase
IR – insulin receptor
IRS – insulin receptor substrate
JNK – c-Jun N-terminal kinase
Keap1 – Kelch-like ECH associated protein
MAPK – mitogen-activated protein kinase
Mas – Mas proto-oncogene, G protein-coupled receptor
MEK – MAPK/ERK kinase
NCoR – nuclear receptor corepressor
MKeR – nuclear receptor corepressor
NF-kB – nuclear factor kappa B
NO – nitric oxide
Nox – NADPH oxidase
Nrf2 – Nuclear factor E2-related factor 2
PDK1 – 3’ phosphoinositide-dependent kinase-1
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