Title: Age- dependent effect of PPARγ agonist pioglitazone on kidney signaling in hypertensive BHR

Running title: PPARγ kidney signaling in hypertension

Linda Grešová1, Miroslava Kvandová1, 2, Peter Kvasnička3, Ima Dovinová1*

1 Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, Sienkiewiczova 1, 813 71 Bratislava, Slovakia.
2 Center for Cardiology, Cardiology I - Laboratory of Molecular Cardiology, University Medical Center of the Johannes Gutenberg-University Mainz, Langenbeckstr. 1, Mainz, Germany.
3 Institute of Particle and Nuclear Physics, Faculty of Mathematics and Physics, Charles University, V Holešovičkách 747/2, Prague, Czech Republic.

*****Corresponding author: Dr. Ima Dovinová, Ima.Dovinova@savba.sk

Correspondence to:
Ima Dovinová, Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, Sienkiewiczova 1, 813 71 Bratislava, Slovak Republik
e-mail: ima.dovinova@savba.sk

1. ABSTRACT

The peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor and nutrition factor which takes part in the cellular signaling by several agonists such as pioglitazone (PIO). PPARγ can serve as potential target in treatments of metabolic syndrome diseases and/or hypertension.

In the present study we investigated the effects of pioglitazone, a PPARγ agonist, on hypertension development in young and adult borderline hypertensive rats (BHR). In renal signaling we observed connections between PPARγ and Nrf2, antioxidant in adult animals and differences between young and adult BHR in Nrf2 activated detoxificant outputs (NQO1, HO-1) and NO-synthases.

Blood pressure in animals had been detected by cuff plethysmography, cell signaling in the kidney was studied by gene expression determination using qPCR and nitric oxide synthase (NOS) activity was measured by radioactive detection.

PIO treatment in adult BHR caused no detectable changes in antioxidant and detoxificant responses. The main effects were observed in blood pressure improvement, endothelial NOS expression and NOS activities in both young and adult BHR.

Keywords: age-dependence, BHR, kidney, PPARγ, Nrf2, NOS, hypertension

2. INTRODUCTION

The peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor and nutrition factor in hypertension (*Tain, Y.L. et al. 2016, Kvandová, M. et al., 2016*). The activity of PPARγ is influenced by a variety of extracellular ligands - glitazones (rosiglitazone, pioglitazone and troglitazone) and intracellular ligands - prostaglandins, leukotrienes, and α-lipoic acid (*Houseknecht, K.L. et al., 2002).*

PPARγ activation takes part in the regulation of lipid metabolism, glucose homeostasis and anti-inflammatory response as well as in several cell signaling pathways (*Polvani, S. et al., 2012*). PPARs undergo a conformational change that causes the translocation to the nucleus and the heterodimerization with another nuclear receptor, the retinoid X receptor (RXR) (*Mello, T. et al., 2009*). The PPAR-RXR heterodimer then binds a DNA portion in the promoter region of target genes, called peroxisome proliferator response element (PPRE), modulating the expression of several genes involved in different physiological or pathological processes (*Kliewer, S.A. et al., 1992).* PPAR*γ* and NRf2 - ARE sytem are connected by a positive feedback. PPAR*γ* may also regulate and modulate other systems, such as the renin - angiotensin system (RAS) and NO-synthases (*Kvandova, M. et al., 2016, 2017*). PPARγ activation may improve dysregulation in metabolic syndrome and hypertension *(Chan, S.H. et al., 2010, Dovinová, I. et al., 2013).*

Blood pressure (BP) regulation is an important and complex process. There are several mechanisms that coordinate BP outputs. Nrf2, which is a redox-sensitive nuclear transcription factor exposed to oxidative stress or noxious attacks. It is involved in the induction of antioxidative, detoxificant, and other cytoprotective genes, which provide protection against oxidative stress induced damage in a variety of cardiovascular diseases and in hypertension (*Majzunova, M. et al., 2013, Barančík, M. et al., 2016).* The positive feedback loop between PPAR*γ* and NRf2 sustains the expression of both transcription factors and their antioxidant genes. Activation of Nrf2 and PPARγ pathways has ameliorating effects on metabolic disorders and injuries caused by oxidative stress *(Lee, C. 2017, Kvandova, M. et al., 2018).* After activation, Nrf2 enters nucleus, binds to the anti-oxidant response element (ARE) and produces NAD(P)H:quinone oxidoreductase-1 (NQO1), heme oxygenase-1 (HO-1), glutathione S-transferase, as well internal antioxidants of different superoxide dismutase (SOD) isoforms *(Li, W. et al., 2008, Lob,H.E. et al., 2010).*

In blood pressure regulation and generally in the cardiovascular system, NO bioavailability plays a protective role in several tissues (vascular tissues, brain stem, kidney). Oral intake of pioglitazone (PPARγ agonist) significantly abrogated adverse molecular events in metabolic syndrome rats and increased NO level in rostral-ventrolateral medulla of brain stem (*Wu, K. et al., 2014*). PPARγ can modulate several signaling pathways: it affects the insulin signaling pathway by modulation of expression and/or phosohorylation of signaling molecules through the PI3/Akt/eNOS pathways (*Kvandová, M. et al. 2017).* Deficiency of endothelial NO-synthase (eNOS) due to endothelial cell dysfunction plays an important role in the pathophysiology of cardiovascular diseases (hypertension, atherosclerosis) as well as in renal injuries *(Nakagawa, T. et al., 2011).* Aging is a universal process that affects all organs. Age-related disruptions in cellular homoeostasis result in decline of organ functions and responsiveness to physiological stress. A gradual decline in renal function occurs in most healthy individuals as they age *(Coresh, J. et al., 2003)*, and the amount of glomerular, vascular and interstitial scarring in the renal tissue of healthy adults increases with age *(Rule, A.D. et al., 2010).*

In the present study we investigated the effects of pioglitazone in the hypertension model of young and adult borderline hypertensive rats (BHR). Our study was focused on changes in blood pressure and on renal signaling in young and adult BHR animals after treatment with PPARγ agonist pioglitazone (PIO). We studied the effects of pioglitazone on blood pressure development and on the components of the antioxidant SOD1-3 and detoxificant NQO1 and HO-1 responses, and on the NOS signaling pathway.

3. MATERIALS and METHODS

3.1. Experimental Design - Animal Model and Treatment Protocol

In our study young (5-weeks old) and adult (12- week old) male borderline hypertensive rats (BHR; offspring of spontaneously hypertensive dams and Wistar-Kyoto sires) were used. The rats were allowed one week to acclimatize to the lab conditions, were housed three per cage: temperature 22–24°C, humidity (45–60%) and with a 12/12 h light/dark cycle. Young and adult BHR animals were divided into two groups: control group (treated with saline solution; $n = 6$) and pioglitazone treatment group (treated with pioglitazone by oral gavage, 10 mg/kg/day during 10 days; $n = 6$). All animal experiments were approved by the Department of Animal Wellness, State Veterinary and Food Administration of the Slovak Republic and carried out in accordance with the Guidelines of the Animal Research and Care Committee of the Institute of Normal and Pathological Physiology of the Slovak Academy of Sciences.

3.2. Blood Pressure Determination

Systolic BP was measured non-invasively by tail cuff plethysmography using a Statham Pressure Transducer P23XL (Hugo Sachs, Germany). All groups of rats (control group and pioglitazone-treated group of young and adult BHR) have been measured between 08:00 A.M. and 11:00 A.M. as described in detail previously *(Puzserova, A. et al., 2013).* BP measurements were performed before, during (every three days), and after the treatment period. Each value was calculated as mean of seven measurements.

3.3. Gene Expression Determination

Expression levels of selected genes were determined by real-time quantitative polymerase chain reaction (RT-qPCR). Total RNA from the renal cortex samples was isolated with TRIsure reagent (Bioline) according to manufacturer's protocol. The isolated total RNA was quantified spectrophotometrically at 260 nm using a Nanodrop 2000 UV-VIS (Thermo

Scientific, USA). The purity of RNA was measured at 260/280 nm (rate ~2.0) and 260/230 (rate range: 1.8-2.2) for elimination of protein, phenol or other contaminants.

cDNA preparation: Reverse transcription reaction was performed using a SensiFAST™ cDNA Synthesis Kit (Bioline, UK) on the Mastercycler Personal (Eppendorf, Germany) according to manufacturer's protocol. Real-time polymerase chain reaction by amplification of cDNA was performed on a CFX96 Real-Time PCR detection system (Bio-Rad, USA) using the Sensi FAST SYBR No ROX kit (Bioline, UK). PCR reaction for each sample was carried out in duplicate for all cDNA. Samples were measured using the Bio-Rad CFX Manager Software (version 2.0). Specific primer pairs were used to amplify the genes studied *(PPARγ, Nrf2, HO-1, NQO1, SOD1, SOD2, SOD3 and eNOS). β-actin* was used as the "housekeeping" gene *(Dovinová, I. et al., 2013, Kvandova, M. et al., 2018).* All chemicals used in this study were purchased from Sigma-Aldrich (Germany), Merck Chemicals (Germany); primers were from Metabion GmbH (Germany).

3.4. Determination of nitric oxide synthase (NOS) activity

Total enzyme activity of NOS was determined in 20% tissue homogenates of the renal cortex using measurement of the conversion of radioactive [^{3}H]-L-arginine (MP Biomedicals, USA) to [^{3}H]-L-citruline *(Bernatova, I. et al., 2002).* The product was detected by a Tri-Carb 2910TR liquid scintillator (PerkinElmer, USA) and results were expressed in pmol/min/mg of tissue proteins, determined by the Lowry method.

3.5 Statistical Analysis

Data are presented by group as mean values ± standard deviation (STD) and the number (n) of independent measurements. BP was analyzed by two-way ANOVA.

Statistical analysis of gene expressions was analysed by one-way ANOVA and unpaired Student's t-test and was carried out using an R language script (*R Development Core Team, 2008).* We carried out two analyses, one for the effect of PIO on gene expression in adult BHR, the other on the effect of PIO on expression of selected genes in adult and young rats. A mixed-factor ANOVA model was fitted to each of the two data sets using the R's *nlme* package (Pinheiro J. et al. 2018), with contribution of individual animals as random factor. For post-hoc comparisons, we used the *glht* method of the *multcomp* R package (*Hothorn T. et al., 2008),* with *single-step* FWER-controlling significance adjustment. Differences were marked as significant for *$p<0.05$ in all tests.

4. RESULTS

4.1. Effect of the PPARγ agonist pioglitazone on blood pressure in BHR animals.

The effects of treatment with the PPARγ agonist pioglitazone significantly (*$p< 0.05$) decreased the final blood pressure (BP). Changes of final BP between the control and PIO groups were 121.5mmHg vs. 115 mmHg in young BHR and 148.5mmHg vs. 136.5 mmHg in adult BHR. The interim BP measurements document a stable BP in young BHR and slightly incrreasing BP in adult BHR control groups. In the PIO groups, BP monotonically and uniformly decreased during the treatment period in both young and adult rats.

4.2. Effect of the PPARγ agonist pioglitazone on Nrf2 outputs in the kidney of BHR.

Nrf2 target genes, HO-1 and phase II-detoxifying gene NQO1, were detected in tested BHR after PIO treatment. PPARγ are activator positively connected with Nrf2 in kidney, which may be the underlying mechanism of its reno-protective effect *(Li, W. et al., 2008).* **Figure 1** shows that in young BHR both Nrf2 signaling outputs in the kidney, HO-1 and NQO1 gene expression, were significantly increased, while no such response was observed in adult BHR.
Pioglitazone treatment and administration markedly elevated PPARγ mRNA and Nrf2 mRNA levels and increased gene expression of SOD1, SOD2, and SOD3 compare to control group in

young BHR (Kvandová M. et al 2018). **Table 1** lists PPARγ, Nrf2, and SOD isoforms gene expressions, which were not significantly changed in adult BHR.

Figure 1. Effect of PIO on Nrf2 outputs in the kidney of young and adult BHR. Effect of pioglitazone on gene expression of HO-1 and NQO1 (mRNA) in the kidney of young and adult BHR. Gene expression was normalized on the "housekeeping" gene, β-actin. Control groups (BHR young-Control: n = 6; BHR adult-Control: n = 5), groups treated with pioglitazone (BHR young-Pioglitazone: n = 6; BHR adult-Pioglitazone: n = 6). Values represent mean ± STD, asterisks indicate statistical significance of PIO versus the corresponding control group (*p < 0.05).

Table 1. BHR adult outputs of gene expressions: PPARγ, Nrf2, and SOD isoforms. "Housekeeping" gene β-actin, BHR adult-Control: n = 5 BHR adult-Pioglitazone: n = 6. Values represent mean ± STD, asterisks indicate statistical significance *p < 0.05 PIO vs. control.

4.3. Effect of the PPARγ agonist pioglitazone on NO-synthases in the kidney of BHR

In the kidney of young BHR, we observed a significant increase of mRNA eNOS as well as NOS activities. The same effect, with yet higher outputs, was observed in the kidney of adult BHR (see **Figure 2**).

Figure 2. PIO effect on NO-synthases in the kidney of BHR. Effect of pioglitazone on A: gene expression of eNOS (mRNA) in the kidney of young and adult BHR; B: NOS kidney activities in young and adult BHR. Gene expression was normalized on the 'housekeeping' gene, β-actin. Control groups (BHR young-Control: n = 6; BHR adult-Control: n = 5), groups treated with pioglitazone (BHR young-Pioglitazone: n = 6; BHR adult-Pioglitazone: n = 6). Values represent mean ± STD, asterisks indicate statistical significance of PIO versus the corresponding control group (*p < 0.05).

5. DISCUSSION

Hypertension is an important facet of the metabolic syndrome. Adulthood hypertension and metabolic syndrome can be programmed in response to nutritional insults in early life. Peroxisome proliferator-activated receptors (PPARs) serve as nutrient-sensing signalling, linking nutritional programming to hypertension and the metabolic syndrome. PPARγ are expressed in the kidney and are involved in blood pressure control (*Tain, Y.L. et al., 2016*). Some studies suggest that administration of a PPARγ agonist (e.g. rosiglitazone) to SHRs may achieve a blood pressure reduction effect by different mechanisms in different stages of hypertension (*Li R. et al., 2010*).

In the highly hypertensive rats, SHR, the PPARγ agonist pioglitazone decreases blood pressure only in young animals (*Dovinová, I. et al., 2013*), but not in adult SHR (*Kvandova, M. et al., 2015*). In the present study of borderline hypertension in BHR, PIO treatment did improve blood pressure: significant BP decrease was observed in both young and adult BHR.

Blood pressure regulation is an important and complex process including a variety of factors, where normal kidney function plays a considerable role. Experimental and physiological evidence indicates that hypertension development may be affected by the renal PPARγ-dependent mechanisms (*Tain, Y.L. et al., 2016*). In our experiments, we observed no increase in PPARγ and Nrf2 expression in the kidney of adult BHR (**Table 1**), while PIO administration markedly elevated PPARγ and Nrf2 mRNA levels in young BHR as has been observed previously in young BHR in a similar study (*Kvandova, M. et al., 2018*). PPARγ-dependent activation resulting in SOD1 and SOD2 upregulation and PPARγ-dependent activation of Nrf2-mediated elevation of SOD3 expression in young BHR has also been reported by other authors *(Zhu, H. et al., 2005, Kvandova M. et al., 2018).* Similar differences between young and adult BHR were also observed in Nrf2 outputs, HO-1 and NQO1 (**Figure 1**).

In the cardiovascular system, eNOS contributes to the regulation of blood flow and blood pressure and in experimental animals deficiency of endothelial nitric oxide synthase

(eNOS$^{-/-}$) produces hypertension with lower circulating nitrite levels (*Wood, K.C. et al., 2013*). Endothelial nitric-oxide synthase (eNOS), an essential enzyme to the maintenance of cardiovascular integrity by producing NO, which is a key molecule with multiple functions, including vasodilation. Therefore, dysregulation of eNOS is thought to contribute to pathogeneses of certain vascular diseases, such as atherosclerosis and hypertension. eNOS is regulated not only at the level of expression, but also non-genomically, by subcellular targeting, protein-protein interactions, fatty acylation, and phosphorylation. Several specific phosphorylation sites responsible for eNOS activation have been identified. In contrast, there is evidence of phosphorylation sites that decrease eNOS activity. It has been shown that treatment with troglitazone increases NO production by a PPARγ-dependent pathway and a PPARγ-independent direct effect on eNOS phosphorylation (*Cho D.H. et al., 2003*). Thiazolidinediones (TZD) – PPARγ agonists – are also known to act in part through AMPK activation. There is growing evidence that AMP-activated protein kinase (AMPK) plays a decisive role in normal renal physiology and pathogenesis of hypertension. (*Tain, Y.L. & Hsu C.N.*, 2018). In the vasculature, activation of endothelial AMPK has been shown to phosphorylate eNOS^{1177}, stimulating NOS activities by NO release and subsequent vasodilatation of both large conduit and resistance arteries (*Fu, J. et al., 2016).* In our experiments on adult BHR we did not observed changes in PPARγ expression, but eNOS expression was increased (**Figure 2A**). eNOS expression can be activated through PPARγ-independent direct effect. It seems that the effect in adult rats was achieved through PIO action. PIO administration in young and adult BHR improves NOS activities (**Figure 2B**). Thus, further examination is required to understand the protective effects of TZDs in programmed hypertension and kidney disease, which are exerted via PPARγ-independent direct effect or via the PPARγ signaling pathway.

Acknowledgments: The study was supported by grants VEGA 2/0148/17 and APVV-038-12.

REFERENCES:

1. Tain, Y.L., Hsu C.N., Chan J.Y. (2016): PPARs Link Early Life Nutritional Insults to Later Programmed Hypertension and Metabolic Syndrome. *Int J Mol Sci,* **17** (1), 20.
2. Kvandová, M., Majzúnová, M., Dovinová, I. (2016): The role of PPARgamma in cardiovascular diseases. *Physiol Res*, ***65,*** Suppl 3, S343-S363.
3. Houseknecht, K.L., Cole B.M., Steele P.J. (2002): Peroxisome proliferator-activated receptor gamma (PPARγ) and its ligands: a review. *Domestic Animal Endocrinology*, **22 (1)**, 1–23.
4. Polvani, S., Tarocchi, M., Galli, A. (2012): PPAR and oxidative stress: Con(β) catenating NRF2 and FOXO. *PPAR Research*, vol. **2012**, Article ID 641087, 15 pages.
5. Mello, T., Polvani, S. & A. Galli,A. (2009). Peroxisome proliferator-activated receptor and retinoic X receptor in alcoholic liver disease. *PPAR Research*, **2009,** Article ID 748174, 11 pages.
6. Kliewer,S:A., Umesono, K., Noonan,D.J., Heyman,R.A. & Evans,R.M. (1992). Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature*, **358** (6389), 771–774.
7. Kvandová, M. & Dovinová, I. (2017): Functioning of the PPARγ and its Effect on Cardiovascular and Metabolic Diseases. *In book: Metabolic syndrome*|www.smgebooks.com, SMgroup, pp. 1-41.
8. Chan, S.H., Wu, K.L., Kung, P.S., Chan, J.Y. (2010): Oral intake of rosiglitazone promotes a central antihypertensive effect via upregulation of peroxisome proliferator-activated receptor-gamma and alleviation of oxidative stress in rostral ventrolateral medulla of spontaneously hypertensive rats. *Hypertension*, **55**(6), 1444-1453.
9. Dovinová, I., Barancik, M., Majzunova, M., Zorad, S., Gajdosechová, L., Gresová, L., Cacanyiova, S., Kristek, F., Balis, P. &Chan, J.Y. (2013): Effects of PPARγ Agonist Pioglitazone on Redox-Sensitive Cellular Signalling in Young Spontaneously Hypertensive Rats. *PPAR Res*, **2013**, 541871.
10. Majzunova, M., Dovinova, I. Barancik, M. & Chan J.Y. (2013): Redox signalling in pathophysiology of hypertension. *Journal of Biomedical Science*, **20**, 69.
11. Barančík, M., Grešová, L., Barteková, M., Dovinová, I. (2016): Nrf2 as a key player of redox regulation in cardiovascular diseases. *Physiol Res*, **65**, Suppl 1, S1-S10.

12. Lee, C. (2017): Collaborative Power of Nrf2 and PPARγ Activators against Metabolic and Drug-Induced Oxidative Injury. *Oxid Med Cell Longev*, **2017**, 1378175.
13. Kvandova, M., Barancik, M., Balis, P., Puzserova, A., Majzunova, M., Dovinova, I. (2018): The peroxisome proliferator-activated receptor gamma agonist pioglitazone improves nitric oxide availability, renin-angiotensin system and aberrant redox regulation in the kidney of pre-hypertensive rats. *J Physiol Pharmacol*, **69**(2), 231-243.
14. Li, W., Khor, T.O., Xu, C., Shen, G., Jeong, W.S., Yu, S., Kong, A.N. (2008): Activation of Nrf2-antioxidant signalling attenuates NFkappaB-inflammatory response and elicits apoptosis. *Biochem Pharmacol*, **76**, 1485–1489.
15. Lob, H. E., Marvar, P.J., Guzik, T.J., Sharma, S., McCann, L.A., Weyand, C., Gordon, F.J., Harrison, D.G. (2010): Induction of hypertension and peripheral inflammation by reduction of extracellular superoxide dismutase in the central nervous system. *Hypertension*, **55**(2), 277-283.
16. Wu, K.L., Chao, Y.M., Tsay, S.J., Chen, C.H., Chan, S.H., Dovinova, I., Chan, J.Y. (2014): Role of nitric oxide synthase uncoupling at rostral ventrolateral medulla in redox-sensitive hypertension associated with metabolic syndrome. *Hypertension*, **64**(4), 815-824.
17. Nakagawa, T., Tanabe, K., Croker, B. P., Johnson, R. J., Grant, M. B., Kosugi, T., Li Q. (2011): Endothelial dysfunction as a potential contributor in diabetic nephropathy. *Nat Rev Nephrol*, **7**, 36–44.
18. Coresh, J., Astor, B.C., Greene, T., Eknoyan, G., Levey, A.S. (2003): Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis*, **41**, 1–12.
19. Rule, A. D., Amer, H., Cornell, L.D., Taler, S.J., Cosio, F.G., Kremers, W.K., Textor, S.C., Stegall, M. D. (2010): The association between age and nephrosclerosis on renal biopsy among healthy adults. *Ann. Intern. Med*, **152**, 561–567.
20. Puzserova, A., Slezak, P., Balis, P. & Bernatova, I. (2013): Long-term social stress induces nitric oxide-independent endothelial dysfunction in normotensive rats. *Stress*, **16**, 331-339.
21. Bernatova, I., Pechanova, O., Babal, P., Kysela, S., Stvrtina, S., Andriantsitohaina, R. (2002): Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. *Am J Phys Heart Circ Phys*, **282**(3), H942–948.
22. R Development Core Team (2008): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org
23. Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team (2018): Nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137, https://CRAN.R-project.org/package=nlme.
24. Hothorn, T., Bretz, F., Westfall, P. (2008): Simultaneous Inference in General Parametric Models. *Biometrical Journal*, **50** (3), 346-363.
25. Li, R., Zhang, H., Wang, W., Wang, X., Huang, Y., Huang, C. & Gao, F. (2010): Vascular insulin resistance in prehypertensive rats: role of PI3-kinase/Akt/eNOS signalling. *Eur J Pharmacol*, **628**, 140-147.
26. Kvandova, M., Kratka, D., Balis, P., Barancik, M., Dovinova, I. (2015): The effect of PPARg agonist - pioglitazone on hypertension and redox regulation in young and adult SHR. *Cardiol Letter*, **24**, 240-243.
27. Zhu, H., Itoh, K., Yamamoto, M., Zweier, J.L., Li, Y. (2005): Role of Nrf2 signaling in regulation of antioxidants and phase 2 enzymes in cardiac fibroblasts: protection against reactive oxygen and nitrogen species-induced cell injury. *FEBS Lett*, **579**, 3029-3036.
28. Wood, K.C., Cortese-Krott, M.M., Kovacic, J.C., Noguchi A., Liu, V.B.,Wang,X., Raghavachari,N., Boehm, M., Kato, G.J.,Malte Kelm,M.& Gladwin, M.T. (2013). Circulating Blood eNOS Contributes to the Regulation of Systemic Blood Pressure and Nitrite Homeostasis *Arterioscler Thromb Vasc Biol*, **33** (8), 10 1161.
29. Cho, D.H, Choi, Y.J., Jo, S.A., & Jo, I. (2004): Nitric oxide production and regulation of endothelial nitric-oxide synthase phosphorylation by prolonged treatment with troglitazone: evidence for involvement of peroxisome proliferator-activated receptor (PPAR) gamma-dependent and PPARgamma-independent signalling pathways. *J Biol Chem*, **279** (4), 2499-506.
30. Tain, Y.L. & Hsu, C.N. (2018): AMP-Activated Protein Kinase as a Reprogramming Strategy for Hypertension and Kidney Disease of Developmental Origin. *Int J Mol Sci*, **19** (6).
31. Fu, J., Han, Y., Wang, J., Liu, Y., Zheng, S., Zhou, L., Jose, P.A., Zeng, C. (2016): Irisin Lowers Blood Pressure by Improvement of Endothelial Dysfunction via AMPK-Akt-eNOS-NO Pathway in the Spontaneously Hypertensive Rat. *J Am Heart Assoc*, **5** (11).

Table 1

Adult BHR mRNA ratio to β -actin	**PPARγ**	**Nrf2**	**SOD1**	**SOD2**	**SOD3**
Control mean ± STD	1.74 ±0.654	1.49 ±0.391	1.16 ±0.285	2.30±0.659	1.23 ±0.523
PIO mean ± STD	1.46±0.401	1.21±0.404	1.44±0.393	1.82±0.659	1.75±0.604

Figure1.

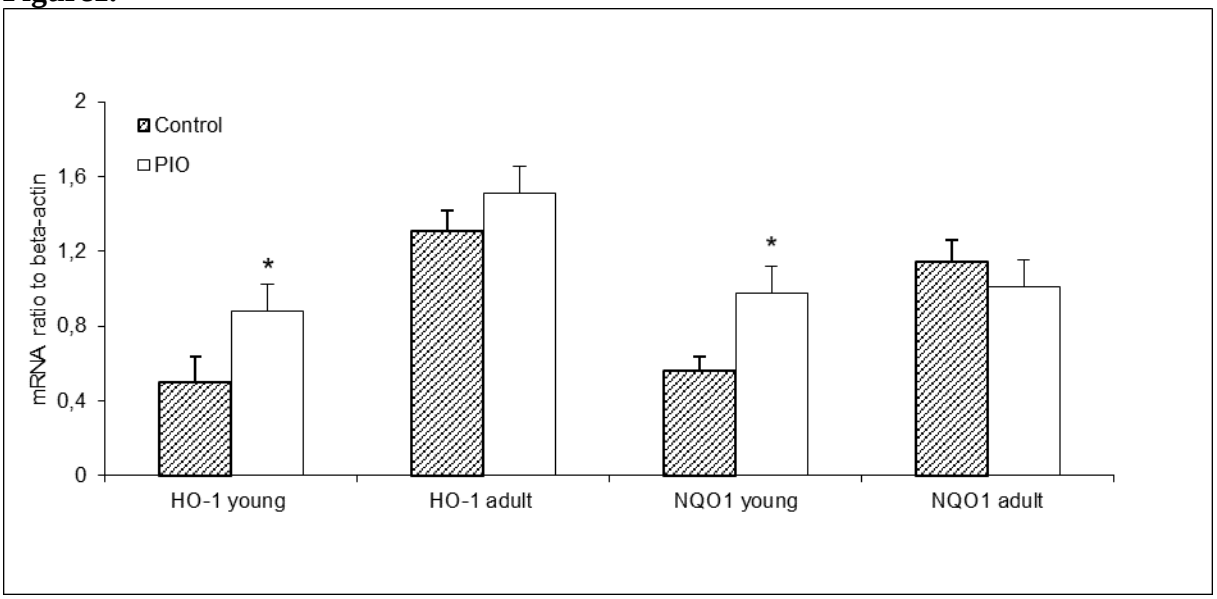

Figure 2A.

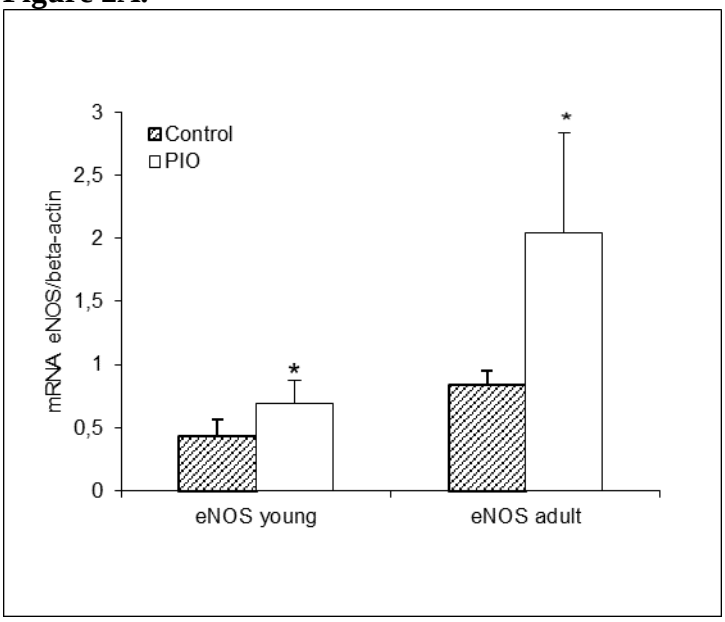

Figure 2B.