Pioglitazone Ameliorates Lipid Metabolic Disorder in KKA^y Mice

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Abstract: Pioglitazone (pio) has been used as an effective hypoglycemic drug in medicine, however, the effects and mechanisms of pio on lipid metabolic disorder are still largely unknown. To explore the effects of pio on serum and liver lipid level and antioxidant ability of mice with lipid metabolic disorder, KKA^y mice were treated with pio for 12 weeks and their lipid and antioxidant indices were compared to those of KKA^y mice without pio treatment. C57BL/6J mice were used as control animals. The results show that pio treatment reduces serum and liver TG, elevates serum HDL-C level, increases serum and liver SOD activity, attenuates serum MDA content, ameliorates liver steatosis, induces liver PPARγ expression and enhances AMPKα phosphorylation level. In conclusion, the results indicate that pio could regulate blood lipid level, reduce liver lipid deposition and enhance antioxidant capacity of mice with lipid metabolic disorder, which is possibly through increasing AMPKα phosphorylation.

Keywords: Pioglitazone, lipid metabolic disorder, AMP-activated protein kinase.

1. INTRODUCTION

With the living standard improving and lifestyle changing, especially the dietary style altering and physical activity decreasing, incidence of non-alcoholic fatty liver disease (NAFLD) has been increasing. NAFLD is pathologically characterized by hepatic lipid deposition and its pathogenesis has not been well understood. Several animal studies have confirmed that thiazolidinediones (TZD) exert antioxidant ability and can prevent atherosclerosis [1]. As a derivative of TZD, pioglitazone (pio) can increase insulin sensitivity, therefore it has been widely used as anti-hyperglycemia agent in clinical medicine [2]. However, the effects and mechanisms of pio on lipid metabolic disorder remain largely unknown.

AMP-activated protein kinase (AMPK) is considered as metabolic energy sensor. It is closely related to insulin resistance and liver lipid content. Studies have suggested that AMPK activation lead to increase fatty acid oxidation in skeleton muscle possibly through activating co-activator 1 of PPARα or PPARγ [3]. Pio can augment AMPK phosphorylation in liver tissue of rats fed with normal diet [4, 5]. Meanwhile, TZD as potent and highly selective PPARγ agonists, can alter transcriptional activity of targeted genes via activation of PPARγ including glucose transporter, insulin receptor and other factors related to energy balance and glucose metabolism [6].

Mouse KKA^y strain is a cross between black KK female mice and yellow male A^y mice. A^y gene not only affects mouse skin color, but also can lead to spontaneous energy metabolism disorder such as obesity, hyperglycemia, hyperlipidemia and hyperinsulinemia under normal diet. KKA^y mouse is widely used as an animal model in the studies of obesity and lipid metabolism while C57BL/6J mouse is used as control animals and considered to be "normal" one [7, 8]. The current study investigated the effects of pio on lipid metabolism and response to oxidative stress of KKA^y mice and explored its possible mechanisms.

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2. MATERIALS AND METHODS

2.1. Materials

Pio hydrochloride was purchased from Jiangsu Hengrui Pharmaceuticals Co., Ltd, China. Assay kits for triglyceride (TG), cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), superoxide dismutase (SOD), and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Institute of Biotechnology, China. Rabbit monoclonal antibodies against PPARγ, AMPKα, and phospho-AMPKα at Thr^{172} were purchased from CST Company, USA. Rabbit monoclonal anti-GAPDH antibody was purchased from Wuhan Boster Company. BCA kit for protein measurement and HRP-conjugated anti-rabbit antibody were purchased from Shanghai Beyotime Company, China.

2.2. Animal Experiment

Twenty four SPF grade KKA^y male mice at age of eight weeks were kindly gifted by Prof. Wenhua Ling (School of Public Health, Sun Yat-sen University). These mice and 12 male C57BL/6J mice from Medical Experimental Animal Center of Guangdong Province were fed with AIN93G basic diet for one week. After collecting their blood samples from orbital veins for measurement of serum lipid-related indices, KKA^y mice were randomly assigned into two groups according to their body weight: 12 mice in pio-untreated group were continuously fed with AIN93G basic diet; the others in pio-treated group were fed with basic diet supplemented with 30 mg pio/kg diet. The pio dose selected in the study was referred to experiments done before, while it had been proved to ameliorate lipid metabolic disorder in *ob*/*ob* mice [9]. Twelve male C57BL/6J mice in control group were fed with basic diet. Mice were kept individually with one per cage. Animals were free to eat and drink water and weighed once a week. Twelve weeks later, all mice were fasted for 12 h. Mice were anesthetized and fasting blood was drawn by eyeball removal. Animals were sacrificed and livers were collected. This study was approved by the Institutional Animal Care and Use Committee at Sun Yat-Sen University.

2.3. Serum Lipid Determination

Serum lipid-related indices such as TG, TC, LDL-C and HDL-C were measured by kits according to the protocols provided by the manufacturer on an automatic biochemical analyzer (Cobas Integra 800, Roche, Switzerland).

2.4. Determination of Lipid-Related Indices in Liver and Liver Index

The liver (500 mg) from each mouse was ground with 0.5 mL of saline and mixed with 4.5 mL of chloroform: methanol (1:1) mixture. After oscillation, the mixture was kept statically overnight at 4 ºC. Supernatant was obtained by centrifugation at 3500 rpm for 20 min and used to determine liver TG and TC levels using kits according to the protocol provided by the manufacturer. Liver index was calculated using the following formula:

$$\text{Liver index (\%)} = [\text{Liver weight (g)}/\text{mouse weight (g)}] \times 100$$

2.5. Determination of SOD Activity and MDA Content

Serum SOD activity and MDA content were measured using kits according to the protocol provided by the manufacturer. Liver was prepared as 10% saline homogenate and centrifuged at 3500 rpm at 4 ºC for 20 min. The supernatant obtained was used to measure liver SOD activity and MDA content by using kits according to the protocols, respectively.

2.6. Liver Biopsy

Fresh liver tissues were used to prepare frozen liver section. Liver tissues were immersed in OCT compound and snap-frozen in liquid nitrogen. The embedded frozen tissues were sectioned as slices with thickness of 4 μm using a cryostat section machine. The obtained frozen slices were placed on slides pre-coated with poly-lysine and stained with oil red O for fat and hematoxylin for nucleus. After sealed with neutral balsam, samples were examined under light microscope and photographed.

2.7. Determination of Total PPARγ, Total AMPKα and Phospho-AMPKα

Proteins of liver tissues were extracted with RIPA (Radio Immunoprecipitation Assay) buffer and quantified using BCA (bicinchoninic acid) kit. Proteins of each sample were separated by 10% SDS-PAGE and transferred onto PVDF (Polyvinylidene Fluoride) membrane. The membrane was then blocked with 5% skim milk at room temperature for 1 h and incubated with 1000-fold diluted antibodies directed against proteins as indicated overnight at 4 ºC. After washed for three times, the membrane was incubated with 5000-fold diluted HRP-labeled secondary antibody at room temperature for 1 h and developed using an

Table 1. Effect of pio on Mice Body Weight

Group	Body Weight (g)				
	0 w	6 w	$BW_{6w} - BW_{0w}$	12 w	$BW_{12w} - BW_{0w}$
Pioglitazone-untreated	35.3±5.8	36.3±4.8	1.1±0.7	37.8±2.8	2.6±1.6
Pioglitazone-treated	35.8±4.9	37.5±2.9	1.7±0.9	40.5±3.1	4.4±2.6
C57BL/6J	28.1±2.4**	29.5±2.5**	1.4±0.4	31.6±1.5$^{**\Delta}$	3.3±1.3

Note: $^{**}P$<0.01 compared with pio-untreated group in respective week while $^{\Delta}P$<0.05 compared with 0 w in each group, n=12.

Table 2. Effect of pio on Mice Serum Lipid Level

Group	TG (mmol/L)		TC (mmol/L)		LDL-C (mmol/L)		HDL-C (mmol/L)	
	0 w	12 w	0 w	12 w	0 w	12 w	0 w	12 w
Pioglitazone-untreated	2.43±0.51	2.60±0.41	2.35±0.42	3.29±0.66$^{\Delta\Delta}$	1.35±0.33	1.48±0.37	2.07±0.35	2.12±0.44
Pioglitazone-treated	2.42±0.45	1.55±0.32$^{**\Delta\Delta}$	2.29±0.42	3.25±0.45$^{\Delta\Delta}$	1.31±0.28	1.32±0.38	2.08±0.35	3.31±0.53$^{**\Delta\Delta}$
C57BL/6J	1.15±0.31**	1.35±0.22**	1.82±0.38	1.88±0.39**	0.85±0.25*	0.95±0.25*	1.85±0.24	1.96±0.35

Note: $^{*}P$<0.05 and $^{**}P$<0.01 compared with pio-untreated group in respective week while $^{\Delta\Delta}P$<0.01 compared with 0 w in each group, n=12.

enhanced chemiluminescence detection system (Amersham Pharmacia, UK).

2.8. Data Analysis

Data were expressed as mean ± standard deviation and analyzed using SPSS 11.0 statistical software. Differences between three groups and between different time (in Table **1** and Table **2**) were analyzed by using repeated-measure ANOVA (General linear model). Differences between groups (in Table **3** and Table **4**) were analyzed using randomized-design one-way ANOVA while differences within groups were compared with LSD method. A *P*-value less than 0.05 or 0.01 was considered as statistical significance.

3. RESULTS

3.1. Effects of pio on Body Weight in KKA^{y} Mice

We first examined the effect of pio on mice body weight. As shown in Table **1**, at the beginning of the experiments, average body weight of KKA^{y} mice was 35~36 g, which is significantly higher than 28 g of the control C57BL/6J mice (P<0.01) as expected. After treated with pio for 6 weeks or 12 weeks, their average body weights were still significantly higher than those of C57BL/6J mice. Interestingly, treatment with pio for 6 weeks and 12 weeks further slightly increased the body weight of KKA^{y} mice, although the differences between the treated and non-treated KKA^{y} mice were not significant (P>0.05).

3.2. Effects of pio on Lipid Level in KKA^{y} Mice

We then studied the effect of pio on mouse serum lipid level. As shown in Table **2**, at the beginning of the experiment, TG and LDL-C levels in KKA^{y} mice were significantly higher than those of the control C57BL/6J mice (P<0.05 and P<0.01, respectively). At the end of the experiment, all the above indices were still higher in KKA^{y} mice (pio-untreated group) compared to the control. However, treatment with pio for 12 weeks significantly decreased serum TG level and increased serum HDL-C level in KKA^{y} mice compared with those

Table 3. Effect of pio on Mice Liver TG, TC and Liver Index

Group	TG (mmol/100g liver)	TC (mmol/100g liver)	Liver index (%)
Pioglitazone-untreated	2.55±0.37	2.95±0.28	6.82±0.42
Pioglitazone-treated	1.70±0.26**	2.52±0.22*	7.08±0.48
C57BL/6J	1.12±0.18**	1.65±0.18**	3.77±0.28**

Note: $^{*}P$<0.05 and $^{**}P$<0.01 compared with pio-untreated group, n=12.

Table 4. Effect of pio on Mouse SOD and MDA in Serum and Liver

Group	Serum		Liver	
	SOD (U/mL)	MDA (nmol/L)	SOD (U/mg protein)	MDA (nmol/mg protein)
Pioglitazone-untreated	35.80±2.75	40.75±2.55	251.50±17.95	3.64±0.63
Pioglitazone-treated	50.61±5.32**	25.44±2.38**	336.35±38.48**	2.91±0.38
C57BL/6J	42.45±5.61*	18.21±1.69**	291.28±21.05	2.07±0.22**

Note: *P<0.05 and **P<0.01 compared with pio-untreated group, n=12.

of non-treated KKA^y (P<0.05 and P<0.01, respectively). Serum levels of TC and LDL-C in KKA^y mice were not significantly changed by pio treatment (P>0.05).

We next explored the effect of pio on mice liver lipid level. As shown in Table **3**, at the end of the experiment, liver TG, TC and liver index in KKA^y mice (pio-untreated group) were significantly higher than those in the control C57BL/6J mice (P<0.05 and P<0.01). However, after pio treatment, liver TG and TC levels were significantly decreased (P<0.05 and P<0.01, respectively) and liver index slightly increased (P>0.05) compared with pio-untreated KKA^y mice.

We further investigated the effect of pio on mouse liver lipid using pathologic histology. As shown in Figure **1**, liver cells from the control C57BL/6J mice were structurally intact showing clear and uniformed cytoplasm and no lipid droplets. By contrast, cells from non-treated KKA^y mice had diffused liver steatosis and obvious fatty infiltration. In comparison, cells from 12-week-pio-treated KKA^y mice only had milder liver steatosis and fat deposition.

3.3. Effects of pio on SOD and MDA Contents in Serum and Liver of KKA^y Mice

We next probed the effects of pio on serum and liver SOD activities and MDA contents. As shown in Table **4**, serum SOD in KKA^y mice (pio-untreated group) was lower than the control C57BL/6J mice (P<0.05), while serum and liver MDA contents were higher than C57BL/6J mice (P<0.01). Treatment with pio significantly enhanced serum and liver SOD activities (P<0.01) and attenuated serum MDA contents (P<0.01). Liver MDA content also slightly decreased after pio treatment (P>0.05).

3.4. Effects of pio on Protein Expression and Phosphorylation of PPARγ and AMPKα in the Liver of KKA^y Mice

To further gain knowledge on the possible mechanisms by which pio exerts its function, we then studied its effects on total PPARγ, total AMPKα and phospho-AMPKα levels in liver. As shown in Figure **2**, total liver PPARγ and AMPKα levels in KKA^y mice were similar to those in C57BL/6J mice. However, phospho-AMPKα in KKA^y mice was significantly lower than that in C57BL/6J mice. Treatment with pio significantly elevated total PPARγ and phospho-AMPKα levels, while it had no effect on total AMPKα.

4. DISCUSSION

Although pio is mainly used as an effective hypoglycemic drug in clinical medicine, it also has a wide range of cardiovascular benefits such as lowering

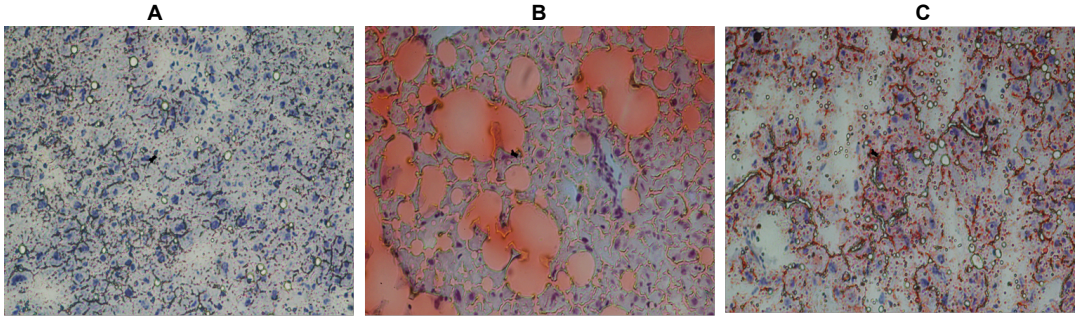

Figure 1. Effects of pio on lipid distribution in mice liver. The livers were stained with oil red O for fat and hematoxylin for nucleus under microscope at 200 x magnification in C57BL/6J (A), KKA^y (B) and KKA^y treated with pio (C).

blood pressure, inhibiting atherosclerosis and improving cardiac remodeling [10, 11]. At present, its effects on improvement of fatty liver have become an extensively studied research area.

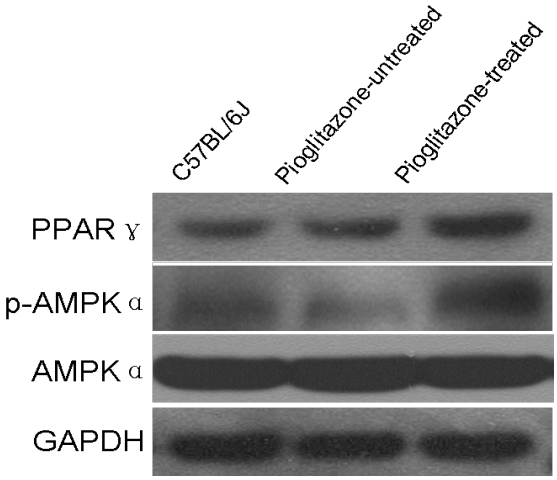

Figure 2. Effects of pio on levels of PPARγ, AMPKα and phospho-AMPKα in mice liver. Proteins of liver tissues were extracted with RIPA buffer and the expression of PPARγ, AMPKα and phospho-AMPKα were assayed with Western blot analysis. Data in each panel are representative of the independent experiment in each group. *n*=6.

Our results showed that KKA^y mice has increased body weight, elevated serum TG, TC and LDL-C as well as attenuated HDL-C compared with the control C57BL/6J mice, indicating that KKA^y mice is a well-established lipid metabolic disorder mouse model. After pio treatment, body weight of KKA^y mice further increased, which is consistent with previous reports that PPARγ agonists can increase body weight in rodents possibly due to increased lipid synthesis [12]. By activating PPARγ, pio promotes differentiation of pre-adipocytes in subcutaneous adipose tissue into adipocytes and apoptosis of visceral mature mast cells, resulting in redistribution of visceral and subcutaneous adipose tissues and increased body weight [13]. Thus, the side effects of pio treatment of diabetes such as weight gain should not be ignored.

As a receptor of insulin sensitizer, it is clear that PPARγ activation can ameliorate lipid metabolic disorder by increasing insulin sensitivity [14]. As a synthetic ligand for PPARγ, pio can activate PPARγ, which further forms a heterodimer with retinoid X receptor (RXR) and translocates to the nucleus, where promoting targeted gene expression [15, 16]. Researches have shown that pio activates target genes downstream of PPARγ, leading to up-regulation of lipoprotein lipase (LPL) and hormone sensitive lipase (HSL) expression, resulting in decrease in the level of plasma TG [17]. Meanwhile, activation of PPARγ can induce expression of adipose-specific factors such as adiponectin to inhibit decomposition of peripheral adipose tissue, lower FFA level and ameliorate liver lipid accumulation [18]. By improving insulin sensitivity of targeted tissues and reducing circulating insulin level, pio can also directly inhibit acyl-CoA synthetase 4 activity in liver cells and inhibit lipid synthesis in liver through PPARγ independent pathways [19]. Our results showed that pio treatment reduced serum and liver TG and liver TC levels, and increased serum HDL-C level and liver index. Treatment of NAFLD patients with pio in combination with vitamin E significantly ameliorated liver steatosis, ballooning degeneration and Mallory bodies [20]. Similarly, our results of liver histopathological examination also showed significantly improved liver fat redistribution in pio treated KKA^y mice.

Lipid metabolic disorder is often associated with oxidative stress. PPARγ ligands can reduce intracellular ROS production by inhibiting NADPH oxidase expression [21]. Studies have shown that pio can inhibit lipid peroxidation in rats with lipid metabolic disorder, increase Cu, Zn-SOD and GPx activities, thus resisting the response of obese rats to oxidative stress [22]. MDA is a product of lipid peroxidation and widely used as index for cell and tissue damage. SOD can effectively remove superoxide anion ($O_2^{\cdot-}$) and its activity has been used as index for body's antioxidant capacity. Our results showed that pio treatment increased serum and liver SOD activity and decreased serum and liver MDA content, indicating that pio has antioxidant ability. However, the relationship between pio's role in improving lipid metabolism and antioxidative effect remains to be elucidated.

AMPK is a member of serine/threonine protein kinase family. The α subunit is essential for its kinase activity. Activation of AMPK can increase lipid metabolism by increasing fatty acid oxidation and reducing triglyceride synthesis. 3-Hydroxy-3-methylglutaric acid (HMG) CoA reductase and acetyl-CoA carboxylase (ACC) are AMPK substrates that play key roles in cholesterol and fatty acid syntheses. Activation of AMPK can increase phosphorylation of HMG-CoA reductase and ACC, resulting in decreased activities of the two enzymes and consequent inhibition of cholesterol and lipid synthesis [23]. In addition, AMPK can decrease malonyl CoA concentration, eventually inducing fatty acid oxidation and inhibiting lipid formation [24]. Our results indicate that pio

increases AMPKα phosphorylation, suggesting that the effects of pio on the improvement of lipid metabolism and antioxidant function may be related to the activation of AMPK. However, its specific mechanism and the possible interaction between AMPK and PPARγ remain unclear.

Taking together, under the current pio dosage in this study, pio could ameliorate serum and liver lipid level and response of KKA^y mice with lipid metabolic disorder to oxidative stress. These effects may be related to its regulation on expression of PPARγ protein and AMPK phosphorylation. However, according to our study, pio treatment may cause weight gain, therefore, the application of appropriate dosage is critical and it should be carefully treated when applied for the prevention of lipid metabolic disorder.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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ABBREVIATIONS

AMP = Adenosine monophosphate

AMPK = AMP-activated protein kinase

MDA = Malondialdehyde

PPAR = Peroxisome proliferator-activated receptor

SOD = Superoxide dismutase

REFERENCES

[1] Lu YX, Zhang Q, Li J, Sun YX, Wang LY, Cheng WM, *et al.* Antidiabetic effects of total flavonoids from Litsea Coreana leve on fat-fed, streptozotocin-induced type 2 diabetic rats. Am J Chin Med 2010; 38: 713-25. http://dx.doi.org/10.1142/S0192415X10008184

[2] Marx N, Duez H, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. Circ Res 2004; 94: 1168-78. http://dx.doi.org/10.1161/01.RES.0000127122.22685.0A

[3] Lee WJ, Kim M, Park HS, Kim HS, Jeon MJ, Oh KS, *et al.* AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARalpha and PGC-1. Biochem Biophys Res Commun 2006; 340: 291-5. http://dx.doi.org/10.1016/j.bbrc.2005.12.011

[4] Lamontagne J, Pepin E, Peyot ML, Joly E, Ruderman NB, Poitout V, *et al.* Pioglitazone acutely reduces insulin secretion and causes metabolic deceleration of the pancreatic beta-cell at submaximal glucose concentrations. Endocrinology 2009; 150: 3465-74. http://dx.doi.org/10.1210/en.2008-1557

[5] Saha AK, Avilucea PR, Ye JM, Assifi MM, Kraegen EW, Ruderman NB. Pioglitazone treatment activates AMP-activated protein kinase in rat liver and adipose tissue in vivo. Biochem Biophys Res Commun 2004; 314: 580-5. http://dx.doi.org/10.1016/j.bbrc.2003.12.120

[6] Komar CM. Peroxisome proliferator-activated receptors (PPARs) and ovarian function--implications for regulating steroidogenesis, differentiation, and tissue remodeling. Reprod Biol Endocrinol 2005; 3: 41. http://dx.doi.org/10.1186/1477-7827-3-41

[7] Castle CK, Colca JR, Melchior GW. Lipoprotein profile characterization of the KKA(y) mouse, a rodent model of type II diabetes, before and after treatment with the insulin-sensitizing agent pioglitazone. Arterioscler Thromb Vasc Biol 1993; 13: 302-9. http://dx.doi.org/10.1161/01.ATV.13.2.302

[8] Saha AK, Kurowski TG, Colca JR, Ruderman NB. Lipid abnormalities in tissues of the KKAy mouse: effects of pioglitazone on malonyl-CoA and diacylglycerol. Am J Physiol 1994; 267: E95-101.

[9] Moritoh Y, Takeuchi K, Asakawa T, Kataoka O, Odaka H. The dipeptidyl peptidase-4 inhibitor alogliptin in combination with pioglitazone improves glycemic control, lipid profiles, and increases pancreatic insulin content in ob/ob mice. Eur J Pharmacol 2009; 602: 448-54. http://dx.doi.org/10.1016/j.ejphar.2008.11.017

[10] Asakawa M, Takano H, Nagai T, Uozumi H, Hasegawa H, Kubota N, *et al.* Peroxisome proliferator-activated receptor gamma plays a critical role in inhibition of cardiac hypertrophy in vitro and in vivo. Circulation 2002; 105: 1240-6. http://dx.doi.org/10.1161/hc1002.105225

[11] Negro R, Dazzi D, Hassan H, Pezzarossa A. Pioglitazone reduces blood pressure in non-dipping diabetic patients. Minerva Endocrinol 2004; 29: 11-7.

[12] Kawai T, Takei I, Oguma Y, Ohashi N, Tokui M, Oguchi S, *et al.* Effects of troglitazone on fat distribution in the treatment of male type 2 diabetes. Metabolism 1999; 48: 1102-7. http://dx.doi.org/10.1016/S0026-0495(99)90122-1

[13] Heikkinen S, Auwerx J, Argmann CA. PPARgamma in human and mouse physiology. Biochim Biophys Acta 2007; 1771: 999-1013.

[14] Chistiakov DA, Potapov VA, Khodirev DS, Shamkhalova MS, Shestakova MV, Nosikov VV. The PPARgamma Pro12Ala variant is associated with insulin sensitivity in Russian normoglycaemic and type 2 diabetic subjects. Diab Vasc Dis Res 2010; 7: 56-62. http://dx.doi.org/10.1177/1479164109347689

[15] Chandra V, Huang P, Hamuro Y, Raghuram S, Wang Y, Burris TP, *et al.* Structure of the intact PPAR-gamma-RXR-nuclear receptor complex on DNA. Nature 2008; 456: 350-6. http://dx.doi.org/10.1038/nature07413

[16] Ondracek CR, Rushing CN, Reese VC, Oropeza CE, McLachlan A. Peroxisome proliferator-activated receptor gamma Coactivator 1alpha and small heterodimer partner differentially regulate nuclear receptor-dependent hepatitis B virus biosynthesis. J Virol 2009; 83: 12535-44. http://dx.doi.org/10.1128/JVI.01623-09

[17] Kageyama H, Hirano T, Okada K, Ebara T, Kageyama A, Murakami T, *et al.* Lipoprotein lipase mRNA in white adipose tissue but not in skeletal muscle is increased by pioglitazone through PPAR-gamma. Biochem Biophys Res Commun 2003; 305: 22-7. http://dx.doi.org/10.1016/S0006-291X(03)00663-6

[18] Krag MB, Nielsen S, Guo Z, Pedersen SB, Schmitz O, Christiansen JS, *et al.* Peroxisome proliferator-activated receptor gamma agonism modifies the effects of growth hormone on lipolysis and insulin sensitivity. Clin Endocrinol 2008; 69: 452-61. http://dx.doi.org/10.1111/j.1365-2265.2008.03231.x

[19] Kim JH, Lewin TM, Coleman RA. Expression and characterization of recombinant rat Acyl-CoA synthetases 1, 4, and 5. Selective inhibition by triacsin C and thiazolidinediones. J Biol Chem 2001; 276: 24667-73. http://dx.doi.org/10.1074/jbc.M010793200

[20] Sanyal AJ, Mofrad PS, Contos MJ, Sargeant C, Luketic VA, Sterling RK, *et al.* A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2004; 2: 1107-15. http://dx.doi.org/10.1016/S1542-3565(04)00457-4

[21] Chen J, Li D, Zhang X, Mehta JL. Tumor necrosis factor-alpha-induced apoptosis of human coronary artery endothelial cells: modulation by the peroxisome proliferator-activated receptor-gamma ligand pioglitazone. J Cardiovasc Pharmacol Ther 2004; 9: 35-41. http://dx.doi.org/10.1177/10742484040090Oi106

[22] El Midaoui A, Wu L, Wang R, de Champlain J. Modulation of cardiac and aortic peroxisome proliferator-activated receptor-gamma expression by oxidative stress in chronically glucose-fed rats. Am J Hypertens 2006; 19: 407-12. http://dx.doi.org/10.1016/j.amjhyper.2005.11.006

[23] Han Y, Wang Q, Song P, Zhu Y, Zou MH. Redox regulation of the AMP-activated protein kinase. PLoS One 2010; 5: e15420. http://dx.doi.org/10.1371/journal.pone.0015420

[24] Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, Yano M, *et al.* Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. Diabetes 2006; 55: 120-7. http://dx.doi.org/10.2337/diabetes.55.01.06.db05-0943

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