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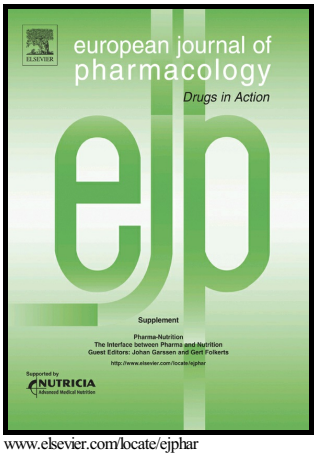

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# A novel PPARα/γ agonist, propane-2-sulfonic acid octadec-9-enyl-amide, ameliorates insulin resistance and gluconeogenesis *in vivo* and *vitro*

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**Abstract**

Peroxisome proliferator-activated receptor alpha/gamma (PPARα/γ) agonists have emerged as important pharmacological agents for improving insulin action. Propane-2-sulfonic acid octadec-9-enyl-amide (N15) is a novel PPARα/γ dual agonist synthesized in our laboratory. The present study investigates the efficacy and safety of N15 on insulin resistance regulation in high fat diet (HFD)-and streptozotocin (STZ)-induced diabetic mice and in palmitic acid (PA)-induced HepG2 cells. Our results showed that N15 remarkably ameliorated insulin resistance and dyslipidemia in vivo, as well as rectified the glucose consumption and gluconeogenesis in vitro. Moreover, the glucose-lowering effect of N15 was associated with PPARγ mediated up-regulation of hepatic glucose consumption and down-regulation of gluconeogenesis. Meanwhile, N15 exerted advantageous effects on glucose and lipid metabolism without triggering weight gain and hepatotoxicity in mice. In conclusion, our data demonstrated that by alleviating glucose and lipid abnormalities, N15 could be used as a potential prophylactic and therapeutic agent against type 2 diabetes and related metabolic disorders.

**Keywords**

propane-2-sulfonic acid octadec-9-enyl-amide; insulin resistance; gluconeogenesis; peroxisome proliferator-activated receptor alpha; peroxisome proliferator-activated receptor gamma

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia, hyperinsulinemia, glucose intolerance and dyslipidemia **(Stumvoll et al., 2005)**. Insulin resistance is caused by the sensitivity to insulin reduction in target tissues, whereas pancreas islet β-cells need to secrete more insulin to maintain euglycemia **(Boden, 2006)**. Besides increasing the risk of T2DM, insulin resistance is a key feature of variety of physiological and metabolic disorders **(Stumvoll et al., 2005)**. Previous studies have shown that the development of insulin resistance can be dependent on liver function, since hepatic glucose production rapidly increases through excessive gluconeogenesis tightly associated with excessive circulating blood glucose **(Barthel and Schmoll, 2003; Nordlie et al., 1999)**.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors involved with various metabolic pathways, such as lipid metabolism, glucose homeostasis and vascular inflammation **(Lee et al., 2003)**. Previous research showed that both PPARα and PPARγ agonists have distinct effects on diabetes treatment **(Kim et al., 2003)**. Activation of PPARα can lead to cellular fatty acid uptake and lipoprotein metabolism, which in turn decreased triglyceride levels and enhanced high-density lipoprotein (HDL) cholesterol levels **(Ferre, 2004)**. Fibrates, a class of hypolipidemic drugs, are ligands of PPARα essential in promoting endocellular fatty acid oxidation **(Chaput et al., 2000)**. PPARγ is the molecular target for thiazolidinediones (TZDs), that due to its ability to promote insulin-sensitizing properties, has been used in the clinical treatment of T2DM

since late 90's **(Yki-Jarvinen, 2004)**. However, pioglitazone is the only molecule from this class still in use for T2DM treatment, while others have been withdrawn from the market due to serious side effects such as hepatotoxicity, heart failure and weight gain **(Cariou et al., 2012)**. These adverse effects have shown to be associated with non-specific ligand activation of PPARγ in diverse tissues **(Argmann et al., 2005; Liu et al., 2015)**. Selective agonists of PPARα have shown to be greatly effective in lipid dysfunction, somewhat reducing side effects induced by PPARγ **(Harrity et al., 2006; Pickavance et al., 2005)**. Meanwhile, some PPARα agonists have also shown to cause certain side-effects that can be avoided by PPARγ activation **(Bateson et al., 1978; Garcia-Romero et al., 1978)**. Therefore, compared to single PPARα or PPARγ agonists, dual PPARα/γ activation has substantially greater advantage in T2DM treatment **(Harrity et al., 2006; Pickavance et al., 2005)**.

Propane-2-sulfonic acid octadec-9-enyl-amide (N15), an analog of oleoylethanolamide (OEA) synthesized in our laboratory, which is resistant to enzymatic hydrolysis. Our previous studies has demonstrated that N15 has potential ability to activate PPARα and PPARγ, due to structural changes (the structure of N15 is shown in Fig. S1) **(Chen et al., 2015)**. In the present study, we investigated the effects of N15 on glucose and lipid metabolism in insulin-resistant models, and revealed the underlying mechanism involving the actions of N15.

## 2. Materials and Methods

### 2.1 Reagents

Streptozotocin (STZ), palmitic acid (PA) and pioglitazone were purchased from Sigma (St. Louis, MO, USA). N15 (purity> 98%) was synthesized in our laboratory. Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS), penicillin and streptomycin were obtained from Gibco BRL (Carlsbad, CA, USA). MK886 (a PPARα inhibitor), T0070907 and GW9662 (PPARγ inhibitors) were provided by Selleck (Houston, TX, USA).

**2.2 Animals**

Male C57BL/6 (6-8 weeks old, weighting 20-22 g) and Kunming mice (KM) (10-11 weeks old, weighting 25-30 g) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All animals were maintained in a specific pathogen-free (SPF) conditions with 12/12-h dark/light cycle environment. All animal studies (including the mice euthanasia procedure) were done in compliance with the regulations and guidelines of Xiamen University institutional animal care and according to the AAALAC and the IACUC guidelines. In addition, maximum efforts were made to minimize pain and suffering of the animals.

**2.3 Drug administration - *in vivo* experiments**

Both male C57BL/6 treated with high fat diet (HFD) added low-dose streptozotocin (STZ) and male KM mice treated with low-dose STZ can be considered as ideal type 2 diabetes animal models with natural disease progression and metabolic characteristics **(Gilbert et al., 2011; Xu et al., 2015)**.

In the present study, C57BL/6 mice were randomly divided into four groups (n=12 for each group): normal, HFD+STZ, HFD+STZ+N15-50 and

HFD+STZ+N15-100, after acclimatized for 1 week. Normal group was fed normal pellet diet (NPD) with 22.47% protein, 12.11% fat and 65.42% carbohydrate and other animal were fed HFD with 30% fat, 20% sugar, 15% protein, 2.5% cholesterol, 1% sodium cholic acid and 31.5% custom carbohydrate. After 6 weeks of dietary manipulation, the HFD feeding mice received an intraperitoneal injection of 35 mg/kg STZ **(Gilbert et al., 2011)**, while the normal group received vehicle (0.1 ml/kg, i.p.). From the next day, these mice were received single daily oral treatment with N15 (50 or 100 mg/kg, respectively, dissolved in Tween-80/ saline, 1/ 99) or vehicle (saline, 1% Tween 80) for 6 weeks.

For inhibition test, KM mice were randomly divided into four groups (n=15 for each group): normal, STZ, STZ+N15-100 and STZ+N15-100+GW9662-1, after acclimatized for 1 week **(Tureyen et al., 2007)**. The normal groups were treated with an intraperitoneal injection of vehicle, while others were treated with STZ (60 mg/kg, i.p.) for five days **(Xu et al., 2015)**. From the next day, these mice were received single daily oral treatment with N15 (100 mg/kg, respectively, dissolved in Tween-80/saline, 1/99) or vehicle (saline, 1% Tween 80) for 6 weeks. GW9662 was given gavage 1 h before administration of N15.

For chronic toxicity test, KM mice were randomly divided into three groups (n=8 for each group): normal, N15 and pioglitazone, after acclimatized for 1 week. From the next day, these mice were received single daily oral treatment with N15 (100 mg/kg, respectively, dissolved in Tween-80/ saline, 1/ 99), pioglitazone (10 mg/kg, respectively, dissolved in Tween-80/ saline, 1/ 99) or vehicle (saline, 1% Tween 80) for 15 weeks.

**2.4 Oral glucose tolerance test**

Oral glucose tolerance test (OGTT) was performed on C57/BL6 mice following overnight-fasting period at the 2$^{nd}$, 4$^{th}$ and 6$^{th}$ week post-treatment. Mice were administered glucose orally at 2.0 g/kg and tail blood samples were obtained at 0, 30, 60 and 120 min post glucose load using glucometer (Johnson, New Jersry, USA) **(Mansuy-Aubert et al., 2013)**.

**2.5 Drug administration - *in vitro* experiments**

HepG2 cells were obtained from the Cell Resource Center of the Shanghai Institutes for Biological Sciences. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1000 mg/l glucose, 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37℃under humidified atmosphere containing 5% $CO_2$ and 95% $O_2$. The cells were subcultured every 2 days until passage. To induce insulin resistance, HepG2 cells were treated with 0.25 mM palmitic acid (PA) dissolved in fatty acid-free bovine serum albumin (BSA), and cultured in DMEM serum starvation medium containing 2% Fetal Bovine Serum (FBS) for 24 h **(Joshi-Barve et al., 2007)**. For biochemical index and protein expression measurement, PA pretreated cells were incubated with medium containing various concentrations of N15 **(Zhao et al., 2016)** or pioglitazone or vehicle for 12 h. The peroxisome proliferators-activated receptors α (PPARα) inhibitor MK886 (20 μM) **(Qin et al., 2007)** and the proliferators-activated receptors γ (PPARγ) inhibitor T0070907 (20 μM) **(Giuliano et al., 2009)** were added into the medium 2 h before administration of N15.

**2.6 Cell viability assay**

To evaluate the toxicity of N15 and pioglitazone respectively on HepG2 cells, the MTS assay was performed. HepG2 cells were plated in 96-well plates ($5 \times 10^3$ cells/ well) and treated with various concentrations of N15 or pioglitazone separately for 6, 12 and 24 h. Consequently, 100 μl MTS solution (5 mg/ml in PBS) was added to each well, and cells were incubated at 37°C for additional 4 h. Absorbance was detected at 490 nm using a Microplate Reader (Molecular Devices, Silicon Valley, CA, USA). Each experiment was run in triplicate. The viability was calculated according to the following formula: *Viability (%)= A490 (sample) / A490 (control)=100%.*

**2.7 Biochemical studies**

Mice were fasted for 8 h, and the fasting blood glucose (FBG) levels were measured using a blood glucose meter (Johnson & Johnson New Brunswick, NJ), once a week. The fasting blood insulin (FIns) level was determined using an ELISA kit (ALPCO, Windham, NH). Insulin resistance was assessed by calculating the homeostatic model assessment of insulin resistance (HOMA-IR), which was previously described. In addition to the serum concentrations of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glutamic-pyruvic transaminase (GPT) and glutamic oxalacetic transaminase (GOT), the level of GPT and GOT in liver tissue were measured by the commercial assay kits (Jiancheng, China) according to the manufacturer's instructions.

**2.8 Glucose consumption assay**

The cells were cultured in 6-well plates ($2\times10^6$ cells/ well) and treated with various concentrations of N15 or pioglitazone in FBS-free DMEM (1000 mg/l

D-glucose) supplemented with 0.25% BSA for 12 h. The left blank, normal (PA-free) and PA group cells were treated with the same BSA DMEM for the same time duration. The glucose concentration in the medium was determined by a glucose oxidase–peroxidase assay kit (Jiancheng, China). The level of glucose consumption was calculated by the glucose concentrations of blank wells subtracting the remaining glucose in cell plated wells. The level of glucose consumption in the normal group (PA-free) was used as a reference to determine the uptake effect **(Yin et al., 2002; Yin et al., 2009)**.

### 2.9 Glucose production assay

The medium in 6-well plates was replaced with 2 ml of FBS and glucose-free DMEM supplemented with 20 mM sodium lactate and 2 mM sodium pyruvate. After 12 h of incubation, half of the glucose production buffer was collected and the glucose concentration was measured using a glucose oxidase–peroxidase assay kit (Jiancheng, China). The values were normalized to the total protein content, determined from the whole-cell extracts **(Lin et al., 2009)**.

### 2.10 Western blot analyses

Proteins were extracted by RIPA lysis buffer (Aidlab Biotechnologies, Beijing, China) containing protease inhibitor (Roche, Basel, Switzerland) and phosphatase inhibitors (Applygen, Beijing, China). After quantification with a BCA protein assay kit (PIERCE, Rockford, USA), proteins were electrophoresed by SDS-PAGE and transferred to a PVDF membrane (Millipore). The 5% Nonfat-Dried milk (Solarbio, Beijing, China) in TBST was used to block PVDF membranes. The membranes were incubated with primary antibodies at 4℃

overnight. Bound antibodies were incubated with HRP-conjugated goat anti-rabbit secondary antibodies (ZSGB-BIO, China) at room temperature for 1 h. The protein bands were visualized and quantified by scanning densitometry using ImageStation 4000R (Rochester, New York, USA).

### 2.11 Statistical analysis

The results were expressed as the mean ± S.E.M. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test for comparisons between two groups followed by Student-Newman-Keuls test (Prism 5 for Windows, GraphPad Software Inc., USA). P values< 0.05 were considered statistically significant.

## 3. Results

### 3.1 The antihyperglycemic and antihyperlipidemic of N15 in diabetic C57BL/6 mice

Several other PPARα/γ dual agonists have shown the ability to ameliorate insulin resistance and hyperlipidemia in diabetic individuals. To examine whether such beneficial effects could be achieved by N15 in vivo, N15 was administered to diabetic C57BL/6 mice model which was induced by HFD and low-dose intraperitoneal injection of STZ. N15 significantly reduced blood glucose in the second week (**Fig. 1A**). After six weeks of treatment with N15, serum insulin (**Fig. 1B**) and HOMA-IR (**Fig. 1C**) were significantly reduced to levels equivalent in non-diabetic mice (first effect was observed at the $4^{th}$ week of treatment) (**Fig. S2A** and **B**). N15 significantly improved glucose tolerance (50

and 100 mg/kg) at 4th and 6th week post-treatment (**Fig. 1E** and **F**). There was a little change in glucose tolerance at the second week (**Fig. 1D**). Moreover, N15 triggered favorable changes in plasma lipids, that were identified by a significant decrease in TC, TG, LDL-C levels (**Fig. 1G, H** and **J**), while there was no effect in the HDL-C level (**Fig. 1I**). Unlike pure PPARγ ligands that have shown to significantly increase body weight, N15 prevented body weight gain in diabetic C57BL/6 mice (Fig. S3). These data indicated that N15 efficiently improves glucose metabolism and lipid contents without causing significant body weight gain.

### 3.2 Pioglitazone induced hepatocyte cells apoptosis

The cytotoxicity of N15 and pioglitazone were evaluated using the MTS assay. Under basic conditions, cell viability was unaffected by N15 with all the used concentrations (20, 50, 100 and 200 μM) over 24 h treatment (**Fig. 2A- C**). However, pioglitazone (100 and 200 μM) treatment caused different cytotoxicity over 12 and 24 h (**Fig. 2A-C**). Moreover, there was a significant dose-dependent increase in expression of apoptosis-related protein B-cell lymphoma-2 associated X protein (Bax) and cleaved cysteine aspartic protease-3 (cleaved caspase-3), and reduced expression of B-cell lymphoma-2 (Bcl-2) after pioglitazone treatment of hepatocytes (**Fig. 2E-G**). In contrast, there was no change in expression of Bcl-2, Bax and cleaved caspase-3 in HepG2 cells after N15 treatment.

To further elucidate the toxicity of N15 and pioglitazone *in vivo*, the levels of GPT and GOT were analyzed following N15 or pioglitazone treatment in KM mice for a duration of 15 weeks. Unlike pioglitazone, N15 treatment did not

cause increase in GPT and GOT plasma levels (**Fig. S4A** and **B**). Moreover, there was no change in GPT and GOT hepatic levels following N15 and pioglitazone treatment (**Fig. S4C** and **D**). These results suggested that N15 exhibits lower toxicity compared to pioglitazone (a pure PPARγ agonist).

### 3.3 Effects of N15 and pioglitazone on glucose consumption and gluconeogenesis in HepG2 cells

To further investigate the beneficial effects of N15 on insulin resistance, glucose consumption was examined in human HepG2 cells. As shown in **Fig. 3A**, glucose consumption was significantly up-regulated in PA-stimulated HepG2 cells following N15 or pioglitazone treatment.

To determine the mechanism of the anti-insulin resistance effect of N15 or pioglitazone, glucose production and protein expression levels were analyzed in insulin-resistant HepG2 cells after N15 or pioglitazone treatment. Both N15 and pioglitazone decreased the glucose production and the expression of key gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) in PA-stimulated HepG2 cells (**Fig. 3C, D** and **Fig. S5A**). The observed effects were more significant following N15 treatment compared to pioglitazone (**Fig. 3C** and **Fig. S5A**).

### 3.4 N15 improvement of insulin resistance is depended on PPARγ

To evaluate the role of the PPARα/γ dual pathways in the anti-insulin resistance effect of N15, the PPARα inhibitor MK886 (20 μM) or the PPARγ inhibitor T0070907 (20 μM) were added to PA-stimulated HepG2 cells respectively, which was then followed by N15 treatment (100 μM) for additional 12 h.

Following the treatment with N15, increase in glucose consumption, an obvious reduction of glucose production and expression of PEPCK and G6Pase were observed following N15 treatment (**Fig. 4A, C, D** and **Fig. S5B**). These beneficial changes in glucose consumption, gluconeogensis and the expression of PEPCK and G6Pase induced by N15 were almost completely reversed by the PPARγ inhibitor T0070907, but not the PPARα inhibitor MK886 (**Fig. 4A, C, D** and **Fig. S5B**). These results indicated that PPARγ are required in order for N15 to have the effect on glucose consumption and gluconeogenesis. Furthermore, MK886 or T0070907 blocked the protein expression of PPARα or PPARγ induced by N15 treatment, respectively (**Fig. 4E, F**). There were no changes in glucose consumption, glucose production and protein expression of PEPCK and G6Pase after N15, MK886 or T0070907 alone.

To investigate whether anti-insulin resistance effects of N15 were specifically mediated by PPARγ, we further employed GW9662 to diabetic mice to measure the levels of FBG, FIns and HOMA-IR. The PPARγ inhibitor GW 9662 (1.0 mg/kg) was pre-received 1 h before N15 administration. The levels of FBG, FIns and HOMA-IR decreased following N15 treatment (Fig. 5A, B and C). These changes were all substantially reversed by GW9662 treatment (Fig. 5A, B and C). Our findings indicated that PPARγ are necessary for the anti-insulin resistance effects of N15 in diabetic mice.

## 4. Discussion

In the present study, our data indicated that N15, a dual-PPARα/γ agonist, is a potent and efficient agent that could improve glucose homeostasis and lipid metabolism in rodent models of diabetes. N15 effectively inhibited

gluconeogenesis, while the mechanism may be through PPARγ pathway. Additionally, unlike pure PPARγ agonists, N15 have favorable effects on hyperlipidemia without causing weight gain and hepatotoxicity.

PPARs are important regulators of lipid and glucose metabolism. The lipid-lowering effect of PPARα agonist leads to liberation of fatty acids, which are taken up and reserved as fat in adipocytes, or metabolized in skeletal muscle **(Ferre, 2004)**. PPARγ controls genes involved in insulin sensitivity restoration, and glucose transport **(Argmann et al., 2005; Liu et al., 2015)**. In addition, synthetic ligands for PPARγ are used in the treatment of type 2 diabetes mellitus because they increase glucose consumption into liver and skeletal muscle cells, which in turn are associated with increase in the expression and translocation of the glucose transporters. Even though PPARγ agonists are potent for ameliorating insulin resistance and glycemia, they have only mild to modest effects on lipid parameters that are accompanied with few adverse effects. In this study, we proved that N15, a novel dual PPARα and PPARγ agonist, is effective in improving the abnormal fasting glucose and glucose tolerance, as well as decreasing hyperinsulinemia and HOMA index in diabetic mice. Previous studies shown that long-term treatment with PPAR-selective agents prevents the diabetes progression in T2DM rodents **(Hofmann et al., 1991; Horikoshi et al., 2000)**. Our data showed that N15 treatment can ameliorate insulin resistance in T2DM mice, which is in line with aforementioned findings (**Oberpichler-Schwenk, 1999**). Intriguingly, diabetic mice treated with N15 did not show any signs of weight gain, which is a common side effect of PPARγ agonists in rodents. However, this beneficial effect is not unique to N15, since it has been reported in

several dual-PPARα/γ agonists, and the underlying molecular mechanism appeared to be largely associated with the activation of PPARα. Indeed, dysregulation of lipid metabolism is often accompanied by systemic destruction of insulin and glucose metabolisms. In this study, N15 significantly reduced serum triglyceride levels, which are metabolized as free fatty acids (FFA) that are an important link between obesity and insulin resistance. According to previous studies, similarly to selective PPARα agonists, dual-PPARα/γ agonists could reduce visceral adiposity development by stimulating PPARα mediated FFA oxidation **(Harrity et al., 2006; Pickavance et al., 2005)**. Moreover, N15 treatment decreased plasma TC and LDL levels, these results suggested that N15 promote cholesterol metabolism in diabetic mice. Our findings support the idea that dual-PPARα/γ agonists can successfully treat dyslipidemia because of preferential affinity to PPARα **(Chen et al., 2009; Jeong et al., 2011; Jung et al., 2017)**. It is highly likely that the effect of N15 on lipid metabolism dysfunction is based on the activation of PPARα. In addition, our previous study has showed that N15 has PPARα agonist activity, with higher affinity than PPARγ **(Chen et al., 2015)**, which implies that N15 could transport extra lipids, thus reinforcing lipid catabolism and resulting in weight gain prevention. In fact, the efficiency differences between pioglitazone and other thiazolidinediones refer to their ability to partially activate PPARα **(Cariou et al., 2012)**. However, they are also limited by a number of side effects, some of which have shown to be potentially dangerous **(Boelsterli and Bedoucha, 2002; Hashimoto et al., 2000)**. PPARγ ligands induced hepatic toxicity is a matter of concern, and therefore the safety effect of N15 in hepatocytes still needs to be clarified.

Increasing evidence has shown that various thiazolidinediones lead to drug-induced liver injury in patients, since the accumulation and retention of bile acids caused apoptosis **(Hashimoto et al., 2000; Rao and Reddy, 2001)**. Furthermore, it has been shown that PPARα agonists cause hepatomegaly in rodents because of peroxisome proliferation **(Evans et al., 2004; Holden and Tugwood, 1999)**. In the present study, the results revealed the ascent of concentrations of GPT and GOT in serum following pioglitazone treatment. Similarly, pioglitazone treatment decrease cell viability in hepatocyte. In contrast, N15 treatment had no influence on the concentrations of GPT or GOT in serum and cell viability *in vitro*. Additionally, the results showed that pioglitazone induced hepatocyte apoptosis through increasing protein expression of Bax and cleaved-caspase-3 and decreasing Bcl-2 expression. However, the expression of apoptosis-related proteins were unaffected by N15 treatment. Additionally, the results also indicted that PPARα mediated effects were not dependent on enlargement of liver size (data not shown). These findings indicate that N15 might be used as a potential therapeutic agent for T2DM eliminating the risk of drug-induced hepatocyte apoptosis.

It is well known that the primary cause of insulin resistance is a comprehensive reduction of glucose disposal rate in peripheral tissues and hepatic glucose output suppression **(Barthel and Schmoll, 2003)**. Glucose output is the result of gluconeogenesis and glycogen breakdown, while the glucose disposal rate is the result of glucose consumption and transportation in peripheral tissues. The key enzymes involved in gluconeogenesis are PEPCK and G6Pase, which are also the major underlying mechanism of PPARγ-mediated insulin sensitization **(Kamon**

**et al., 2003)**. In the present study N15 enhanced hepatic glucose consumption, which can be employed in various ways to improve insulin sensitivity. The results were similar to those previously reported in relation to other insulin-resistant models by dual agonists such as SN158, DGF and aleglitazar **(Benardeau et al., 2009; Jung et al., 2017; Lee et al., 2016)**. Increased insulin sensitivity in tissue is serious condition that inhibits gluconeogenesis by suppressing the expression of the key gluconeogenic enzymes, i.e. PEPCK and G6Pase. Compared with vehicle-treated HepG2 cells, the glucose production and the protein expressions of PEPCK and G6Pase were significantly reduced by N15 or pioglitazone treatment. The properties of N15 in reducing gluconeogenesis were stronger compared with pioglitazone, which suggested that N15 might be more effective in inhibiting hepatic glucose production and suppressing insulin resistance.

It has been known that a dual PPARα/γ agonist always includes therapeutic advantages of PPARα and/or PPARγ agonists in metabolic disturbances. As revealed by previous studies, a number of dual PPARα/γ agonists have shown beneficial effects on glucose and lipid metabolisms through tissue-specific activation of PPARα and PPARγ. For instance, CG301269 and P633H, have shown the ability to up-regulate the target genes of PPARα in liver and PPARγ in adipose tissues, respectively **(Chen et al., 2009; Jeong et al., 2011)**. However, there was no direct evidence demonstrating that the major underlying mechanism in improvement of dyslipidemia or pathoglycemia is based on PPARα and/or PPARγ activation or another target gene. Meanwhile, previous findings have shown that several dual PPARα/γ agonists are effective in ameliorating insulin

resistance and lipid parameter, nevertheless they did not show whether they interact or act independently. In addition, just like N15, numerous PPARα/γ agonist have shown to be effective in promoting glucose consumption and suppressing gluconeogenesis. Notably, in the present study, N15 treatment markedly increased the expressions of PPARα and PPARγ in hepatocyte. To verify whether the PPARα and PPARγ signaling pathways were involved in the role of N15 on lipid and glucose metabolism, both PPARα and PPARγ antagonists, MK886 and T0070907, were applied to HepG2 cells. The results showed that N15 markedly promoted glucose consumption, as well as significantly inhibited glucose production and the expression of PEPCK and G6Pase. These changes were almost reversed following pretreatment with T0070907, but not MK886. Meanwhile, the levels of glucose consumption remained unchanged following treatment with MK886 or T0070907, which indicated that MK886 and T0070907 alone could not block the activation of glycometabolism. To further clarify whether PPARγ were major factors of N15 in the treatment of insulin resistance, PPARγ antagonists GW9662 were given to diabetic mice before N15 administration. The results showed that N15 markedly ameliorated the increased levels of FBG, FIns and HOMA-IR, whereas the role of N15 in the regulation of insulin resistance was abolished by GW9662. Consistent with *in vitro* experiments, these results indicated that the anti-insulin resistance effect of N15 may be depended on PPARγ pathway. Taken together, these results demonstrated that PPARγ might play a crucial role in the anti-gluconeogenic and anti-hyperglycemic regulation of N15 on glycometabolism. Unlike other known dual-PPARα/γ agonists, N15 partially activated PPARγ with lower affinity than PPARα. We speculate that the

anti-hyperlipidmia effects of N15 may be mediated by PPARα pathway. Nevertheless, particular molecular mechanism(s) by which N15 would cause increase in adiposity and serum lipid still need to be elucidated.

## 5. Conclusion

As a novel PPAR α/γ agonist, N15 exerts advantageous effects on glucose and lipid metabolism without causing serious side effects detected in existing PPARγ agonists. These roles may be due to its beneficial effects on PPARγ mediated inhibition of gluconeogensis. Therefore, our results suggested that N15 may be a potential and effective compound against type 2 diabetes.

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## References

Andrulionyte, L., Kuulasmaa, T., Chiasson, J.L., Laakso, M., 2007. Single nucleotide polymorphisms of the peroxisome proliferator-activated receptor-alpha gene (PPARA) influence the conversion from impaired glucose tolerance to type 2 diabetes: the STOP-NIDDM trial. Diabetes 56, 1181-1186.

Argmann, C.A., Cock, T.A., Auwerx, J., 2005. Peroxisome proliferator-activated receptor gamma: the more the merrier? European journal of clinical investigation 35, 82-92; discussion 80.

Barthel, A., Schmoll, D., 2003. Novel concepts in insulin regulation of hepatic gluconeogenesis. American journal of physiology. Endocrinology and metabolism 285, E685-692.

Bateson, M.C., Maclean, D., Ross, P.E., Bouchier, I.A., 1978. Clofibrate therapy and gallstone induction. The American journal of digestive diseases 23, 623-628.

Benardeau, A., Benz, J., Binggeli, A., Blum, D., Boehringer, M., Grether, U., Hilpert, H., Kuhn, B., Marki, H.P., Meyer, M., Puntener, K., Raab, S., Ruf, A., Schlatter, D., Mohr, P., 2009. Aleglitazar, a new, potent, and balanced dual PPARalpha/gamma agonist for the treatment of type II diabetes. Bioorganic & medicinal chemistry letters 19, 2468-2473.

Boden, G., 2006. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. Current diabetes reports 6, 177-181.

Boelsterli, U.A., Bedoucha, M., 2002. Toxicological consequences of altered peroxisome proliferator-activated receptor gamma (PPARgamma) expression in the liver: insights from models of obesity and type 2 diabetes. Biochemical pharmacology 63, 1-10.

Cariou, B., Charbonnel, B., Staels, B., 2012. Thiazolidinediones and PPARgamma agonists: time for a reassessment. Trends in endocrinology and metabolism: TEM 23, 205-215.

Chaput, E., Saladin, R., Silvestre, M., Edgar, A.D., 2000. Fenofibrate and rosiglitazone lower serum triglycerides with opposing effects on body weight. Biochemical and biophysical research communications 271, 445-450.

Chen, C.X., Yang, L.C., Xu, X.D., Wei, X., Gai, Y.T., Peng, L., Guo, H., Hao, Z., Wang, Y.Q., Jin, X., 2015. Effect of propane-2-sulfonic acid octadec-9-enyl-amide on the expression of adhesion molecules in human umbilical vein endothelial cells. European journal of pharmacology 756, 15-21.

Chen, W., Zhou, X.B., Liu, H.Y., Xu, C., Wang, L.L., Li, S., 2009. P633H, a novel dual agonist at peroxisome proliferator-activated receptors alpha and gamma, with different anti-diabetic effects in db/db and KK-Ay mice. British journal of pharmacology 157, 724-735.

Evans, R.M., Barish, G.D., Wang, Y.X., 2004. PPARs and the complex journey to obesity. Nature medicine 10, 355-361.

Ferre, P., 2004. The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. Diabetes 53 Suppl 1, S43-50.

Flavell, D.M., Ireland, H., Stephens, J.W., Hawe, E., Acharya, J., Mather, H., Hurel, S.J., Humphries, S.E., 2005. Peroxisome proliferator-activated receptor alpha gene variation influences age of onset and progression of type 2 diabetes. Diabetes 54, 582-586.

Garcia-Romero, E., Lopez-Cantarero, M., Arcelus, I.M., 1978. Dissolution of human gallstone with clofibrate. The Journal of surgical research 24, 62-64.

Gilbert, E.R., Fu, Z., Liu, D., 2011. Development of a nongenetic mouse model of type 2 diabetes. Experimental diabetes research 2011, 416254.

Giuliano, M., Pellerito, O., Portanova, P., Calvaruso, G., Santulli, A., De Blasio, A., Vento, R., Tesoriere, G., 2009. Apoptosis induced in HepG2 cells by the synthetic cannabinoid WIN: involvement of the transcription factor PPARgamma. Biochimie 91, 457-465.

Harrity, T., Farrelly, D., Tieman, A., Chu, C., Kunselman, L., Gu, L., Ponticiello, R., Cap, M., Qu, F., Shao, C., Wang, W., Zhang, H., Fenderson, W., Chen, S., Devasthale, P., Jeon, Y., Seethala, R., Yang, W.P., Ren, J., Zhou, M., Ryono, D., Biller, S., Mookhtiar, K.A., Wetterau, J., Gregg, R., Cheng, P.T., Hariharan, N., 2006. Muraglitazar, a novel dual (alpha/gamma) peroxisome proliferator-activated receptor activator, improves diabetes and other metabolic abnormalities and preserves beta-cell function in db/db mice. Diabetes 55, 240-248.

Hashimoto, T., Cook, W.S., Qi, C., Yeldandi, A.V., Reddy, J.K., Rao, M.S., 2000. Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. The Journal of biological chemistry 275, 28918-28928.

Hofmann, C., Lorenz, K., Colca, J.R., 1991. Glucose transport deficiency in diabetic animals is corrected by treatment with the oral antihyperglycemic agent pioglitazone. Endocrinology 129, 1915-1925.

Holden, P.R., Tugwood, J.D., 1999. Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. Journal of molecular endocrinology 22, 1-8.

Horikoshi, H., Hashimoto, T., Fujiwara, T., 2000. Troglitazone and emerging glitazones: new avenues for potential therapeutic benefits beyond glycemic control. Progress in drug research. Fortschritte der Arzneimittelforschung. Progres des recherches pharmaceutiques 54, 191-212.

Jeong, H.W., Lee, J.W., Kim, W.S., Choe, S.S., Kim, K.H., Park, H.S., Shin, H.J., Lee, G.Y., Shin, D., Lee, H., Lee, J.H., Choi, E.B., Lee, H.K., Chung, H., Park, S.B., Park, K.S., Kim, H.S., Ro, S., Kim, J.B., 2011. A newly identified CG301269 improves lipid and glucose metabolism without body weight gain through activation of peroxisome proliferator-activated receptor alpha and gamma. Diabetes 60, 496-506.

Joshi-Barve, S., Barve, S.S., Amancherla, K., Gobejishvili, L., Hill, D., Cave, M., Hote, P., McClain, C.J., 2007. Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. Hepatology (Baltimore, Md.) 46, 823-830.

Jung, Y., Cao, Y., Paudel, S., Yoon, G., Cheon, S.H., Bae, G.U., Jin, L.T., Kim, Y.K., Kim, S.N., 2017. Antidiabetic effect of SN158 through PPARalpha/gamma dual activation in ob/ob mice. Chemico-biological interactions 268, 24-30.

Kamon, J., Yamauchi, T., Terauchi, Y., Kubota, N., Kadowaki, T., 2003. [The mechanisms by which PPARgamma and adiponectin regulate glucose and lipid metabolism]. Nihon yakurigaku zasshi. Folia pharmacologica Japonica 122, 294-300.

Kim, H., Haluzik, M., Asghar, Z., Yau, D., Joseph, J.W., Fernandez, A.M., Reitman, M.L., Yakar, S., Stannard, B., Heron-Milhavet, L., Wheeler, M.B., LeRoith, D., 2003. Peroxisome proliferator-activated receptor-alpha agonist treatment in a transgenic model of type 2 diabetes reverses the lipotoxic state and improves glucose homeostasis. Diabetes 52, 1770-1778.

Krebs, M., Roden, M., 2005. Molecular mechanisms of lipid-induced insulin resistance in muscle, liver and vasculature. Diabetes, obesity & metabolism 7, 621-632.

Lee, C.H., Olson, P., Evans, R.M., 2003. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. Endocrinology 144, 2201-2207.

Lee, W., Yoon, G., Kim, M.C., Kwon, H.C., Bae, G.U., Kim, Y.K., Kim, S.N., 2016. 5,7-Dihydroxy-6-geranylflavanone improves insulin sensitivity through PPARalpha/gamma dual activation. International journal of molecular medicine 37, 1397-1404.

Lin, Y.Y., Lu, J.Y., Zhang, J., Walter, W., Dang, W., Wan, J., Tao, S.C., Qian, J., Zhao, Y., Boeke, J.D., Berger, S.L., Zhu, H., 2009. Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. Cell 136, 1073-1084.

Liu, C., Feng, T., Zhu, N., Liu, P., Han, X., Chen, M., Wang, X., Li, N., Li, Y., Xu, Y., Si, S., 2015. Corrigendum: Identification of a novel selective agonist of PPARgamma with no promotion of adipogenesis and less inhibition of osteoblastogenesis. Scientific reports 5, 12185.

Mansuy-Aubert, V., Zhou, Q.L., Xie, X., Gong, Z., Huang, J.Y., Khan, A.R., Aubert, G., Candelaria, K., Thomas, S., Shin, D.J., Booth, S., Baig, S.M., Bilal, A., Hwang, D., Zhang, H., Lovell-Badge, R., Smith, S.R., Awan, F.R., Jiang, Z.Y., 2013. Imbalance between neutrophil elastase and its inhibitor alpha1-antitrypsin in obesity alters insulin sensitivity, inflammation, and energy expenditure. Cell metabolism 17, 534-548.

Nordlie, R.C., Foster, J.D., Lange, A.J., 1999. Regulation of glucose production by the liver. Annual review of nutrition 19, 379-406.

Oberpichler-Schwenk, H., 1999. [Type II diabetes mellitus. Rosiglitazone is effective against insulin resistance]. Medizinische Monatsschrift fur Pharmazeuten 22, 288-289.

Pickavance, L.C., Brand, C.L., Wassermann, K., Wilding, J.P., 2005. The dual PPARalpha/gamma agonist, ragaglitazar, improves insulin sensitivity and metabolic profile equally with pioglitazone in diabetic and dietary obese ZDF rats. British journal of pharmacology 144, 308-316.

Qin, S., Liu, T., Kamanna, V.S., Kashyap, M.L., 2007. Pioglitazone stimulates apolipoprotein A-I production without affecting HDL removal in HepG2 cells: involvement of PPAR-alpha. Arteriosclerosis, thrombosis, and vascular biology 27, 2428-2434.

Rao, M.S., Reddy, J.K., 2001. Peroxisomal beta-oxidation and steatohepatitis. Seminars in liver disease 21, 43-55.

Stumvoll, M., Goldstein, B.J., van Haeften, T.W., 2005. Type 2 diabetes: principles of pathogenesis and therapy. Lancet (London, England) 365, 1333-1346.

Tureyen, K., Kapadia, R., Bowen, K.K., Satriotomo, I., Liang, J., Feinstein, D.L., Vemuganti, R., 2007. Peroxisome proliferator-activated receptor-gamma agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents. Journal of neurochemistry 101, 41-56.

Watkins, P.B., 2005. Idiosyncratic liver injury: challenges and approaches. Toxicologic pathology 33, 1-5.

Xu, W., Zhou, Q., Yin, J.J., Yao, Y., Zhang, J.L., 2015. Anti-diabetic effects of polysaccharides from Talinum triangulare in streptozotocin (STZ)-induced type 2 diabetic male mice. International journal of biological macromolecules 72, 575-579.

Yin, J., Hu, R., Chen, M., Tang, J., Li, F., Yang, Y., Chen, J., 2002. Effects of berberine on glucose metabolism in vitro. Metabolism: clinical and experimental 51, 1439-1443.

Yin, J., Zuberi, A., Gao, Z., Liu, D., Liu, Z., Ye, J., 2009. Shilianhua extract inhibits GSK-3beta and promotes glucose metabolism. American journal of physiology. Endocrinology and metabolism 296, E1275-1280.

Yki-Jarvinen, H., 2004. Thiazolidinediones. The New England journal of medicine 351, 1106-1118.

Zhao, Y., Yan, L., Luo, X.M., Peng, L., Guo, H., Jing, Z., Yang, L.C., Hu, R., Wang, X., Huang, X.F., Wang, Y.Q., Jin, X., 2016. A novel PPARalpha agonist propane-2-sulfonic acid octadec-9-enyl-amide inhibits inflammation in THP-1 cells. European journal of pharmacology 788, 104-112.



## Figure legends

### Figure 1

Effect of N15 on glucose and lipid mediation in insulin-resistant C57BL/6 mice. N15 treated groups were received single daily oral treatment with N15 (50 or 100

mg/kg), while the normal and HFD+STZ groups were treated with vehicle at daily basis. (A) Sequential monitoring of blood glucose after 8 h fasting. (B) Fasting blood insulin, and (C) levels of HOMA-IR were measured at 6 weeks post-treatment (D) 2, 4 and 6 weeks post-treatment, OGTT was performed after 8 h fasting. (E-H) The levels of TC, TG, HDL and LDL were measured at 6 weeks post-treatment, respectively. Values are shown as means ± S.E.M (n=12 per group). $^{\#}$ P < 0.05, $^{\#\#}$ P < 0.01, $^{\#\#\#}$ P < 0.001 vs. normal group; $^{*}$ P < 0.05, $^{**}$ P < 0.01, $^{***}$ P < 0.001 vs. HFD+STZ group.

**Figure 2**

Effects of N15 and pioglitazone on cell viability and Bcl-2, Bax and cleaved-caspase-3 protein expresssion in HepG2 cells. HepG2 cells were cultured with different concentrations of N15 (20, 50, 100 and 200 μM) or pioglitazone (20, 50, 100 and 200 μM) for 6, 12, 24 h, respectively. (A-C) Cell viability in different concentrations of N15 or pioglitazone with 6, 12, 24 h treatment was measured by MTS. (D) Representative western blots images of Bcl-2, Bax and cleaved-caspase-3 proteins of HepG2 cells cultured in the absence or presence of different concentrations of N15 or pioglitazone for 12 h. (E-G) Quantitative analysis of Bcl-2, Bax and cleaved-caspase-3. The values are expressed as percentages compared with the normal group (set to 100%) and represented as means ± S.E.M of five separate experiments performed in duplicate (n = 5). $^{\#}$ P < 0.05, $^{\#\#}$ P < 0.01 vs. normal group, $^{\wedge}$ p < 0.05, $^{\wedge\wedge}$ P < 0.01, $^{\wedge\wedge\wedge}$ P < 0.001 vs. N15 group.

**Figure 3**

Effects of N15 and pioglitazone on glucose consumption and the protein expression of key hepatic glucogenesis enzymes in PA-stimulated HepG2 cells. N15 or pioglitazone group were treated with different concentrations of N15 (20, 50 and 100 μM) or pioglitazone (100 μM), while the normal and PA groups were treated with vehicle at daily basis. (A) The levels of glucose consumption. (B) Representative western blots images of PEPCK and G6Pase proteins. (C, D) Quantitative analysis of PEPCK and G6Pase proteins. The values are expressed as percentages compared to normal group (set to 100%) and are represented as means ± S.E.M of five separate experiments performed in duplicate (n = 5). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. normal group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. PA group; $^{\wedge}p < 0.05$ vs. N15-100 group.

**Figure 4**

Effects of PPARα or PPARγ pathways on the anti-insulin resistance action of N15. HepG2 cells were subjected to the PPARα antagonist MK886 (20 μM) or the PPARγ antagonist T0070907 (20 μM) for 2 h, followed by N15 (100 μM) treatment for an additional 12h. (A) The levels of glucose consumption. (B) Representative western blots images of PEPCK, G6Pase, PPARα and PPARγ proteins. (C-F) Quantitative analysis of PEPCK, G6Pase, PPARα and PPARγ proteins. The values are expressed as percentages compared with the normal group (set to 100%) and represented as means ± S.E.M of five separate experiments performed in duplicate (n = 5). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. normal group; $^{**}P < 0.01$, $^{***}P < 0.001$ vs. PA group; $^{\wedge}P < 0.05$, $^{\wedge\wedge\wedge}P < 0.001$ vs. N15-100 group.

**Figure 5**

Effects of PPARγ pathways on the anti-insulin resistance action of N15 in diabetic KM mice. N15 treated groups were received single daily oral treatment with N15 (100 mg/kg) for 6 weeks. N15+GW9662 groups received single daily oral treatment with GW9662 (1.0 mg/kg) 1 h before N15 (100 mg/kg) administration for 6 weeks. The normal and STZ groups were treated with vehicle at daily basis. (A) Sequential monitoring of blood glucose after 8 h fasting; (B) Serum insulin and (C) HOMA-IR was measured at 6 weeks post-treatment. Values are means ± S.E.M (n=15 per group). $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. normal group; $^{*}P < 0.05$, $^{***}P < 0.001$ vs. STZ group; $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ vs. N15 group.

**Supplementary data**

**Figure S1**

The structure of the dual PPARα/γ agonist N15 (propane-2-sulfonic acid octadec-9-enyl-amide).

**Figure S2**

N15 treatment improved insulin resistance in diabetic C57BL/6 mice. N15 treated groups were received single daily oral treatment with N15 (50 or 100 mg/kg), while the normal and HFD+STZ groups were treated with vehicle at daily basis. (A) Serum insulin and (B) HOMA-IR was measured at 2 or 4 weeks post-treatment. Values are means ± S.E.M (n=12 per group). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. normal group; $^{**}P < 0.01$, $^{***}P < 0.001$ vs. HFD+STZ group.

**Figure S3**

Effect of N15 on body weight in insulin-resistant C57BL/6. N15 treated groups were received single daily oral treatment with N15 (50 or 100 mg/kg), while the normal and HFD+STZ groups were treated with vehicle at daily basis. Body weight was measured at 6 weeks post-treatemnt. Values are means ± S.E.M (n=12 per group). $^{\#}$ P < 0.05 vs. normal group; $^{*}$ P < 0.05 vs. HFD+STZ group.

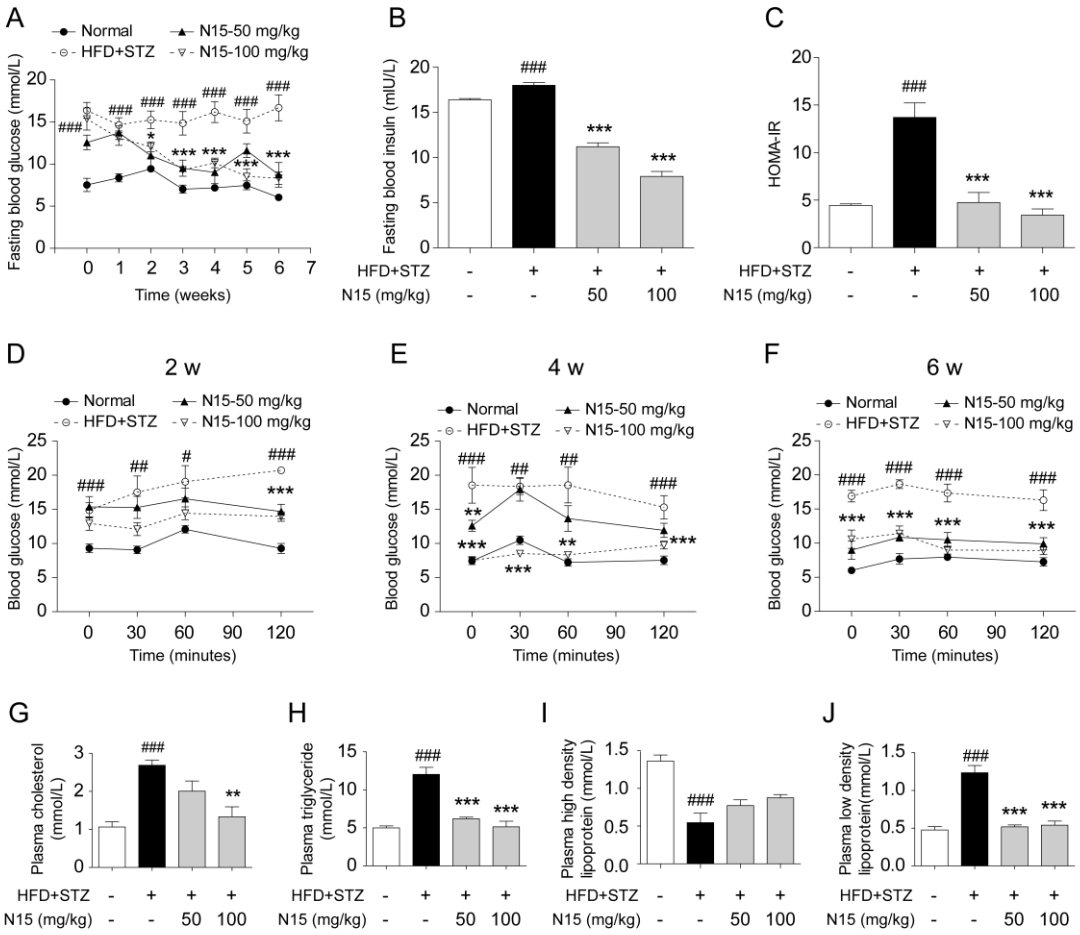
**Figure S4**

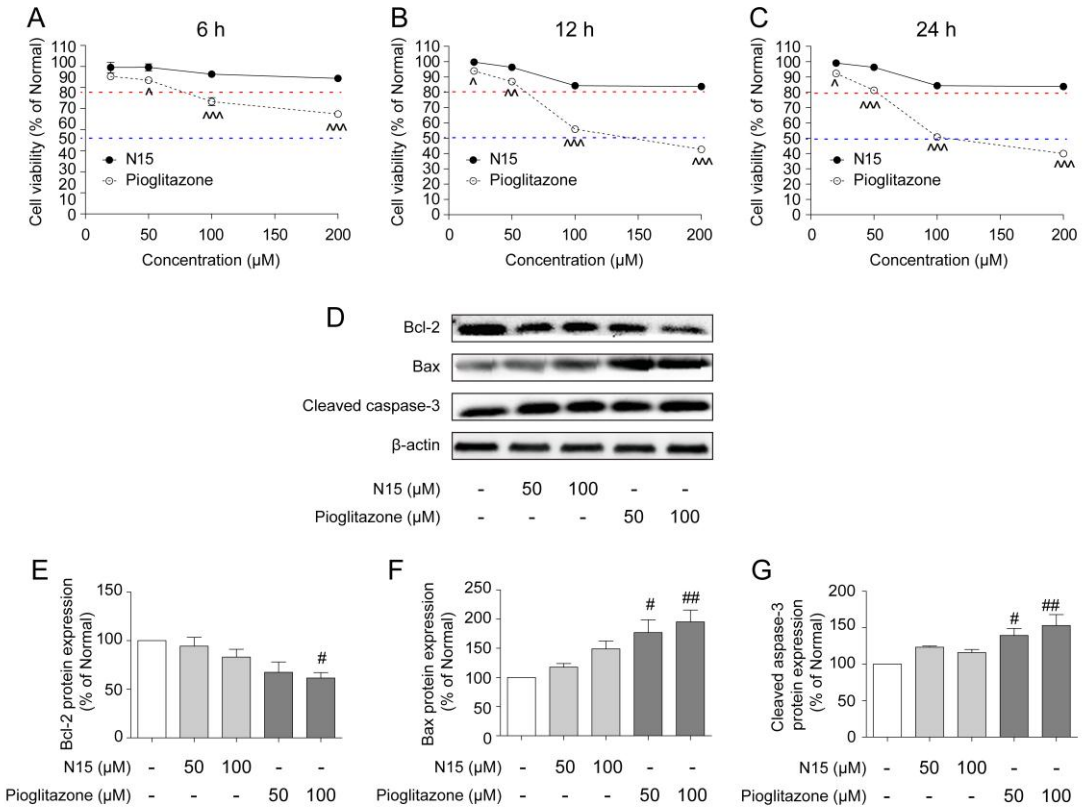
The toxicity of N15 and pioglitazone *in vivo*. N15 or pioglitazone groups were received single daily oral treatment with N15 (100 mg/kg) or pioglitazone (10 mg/kg) for 15 weeks, while normal groups were treated with vehicle at daily basis. (A) Levels of GPT and (B) GOT in serum, and (C) levels of GPT and (D) GOT in liver were measured at 15 week post-treatment. The values are represented as means ± S.E.M (n=8 per group). $^{\#}$ P < 0.05, $^{\#\#}$ P < 0.01 vs. normal group; $^{\wedge\wedge\wedge}$ P < 0.001 vs. N15-100 group.

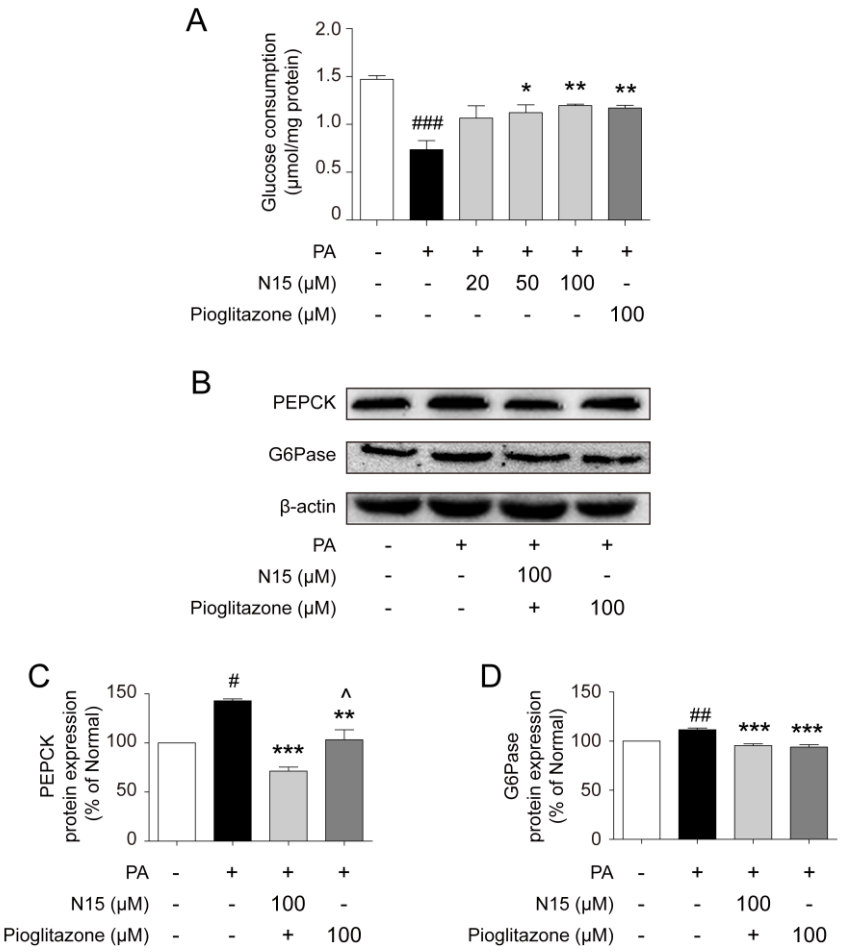
**Figure S5**

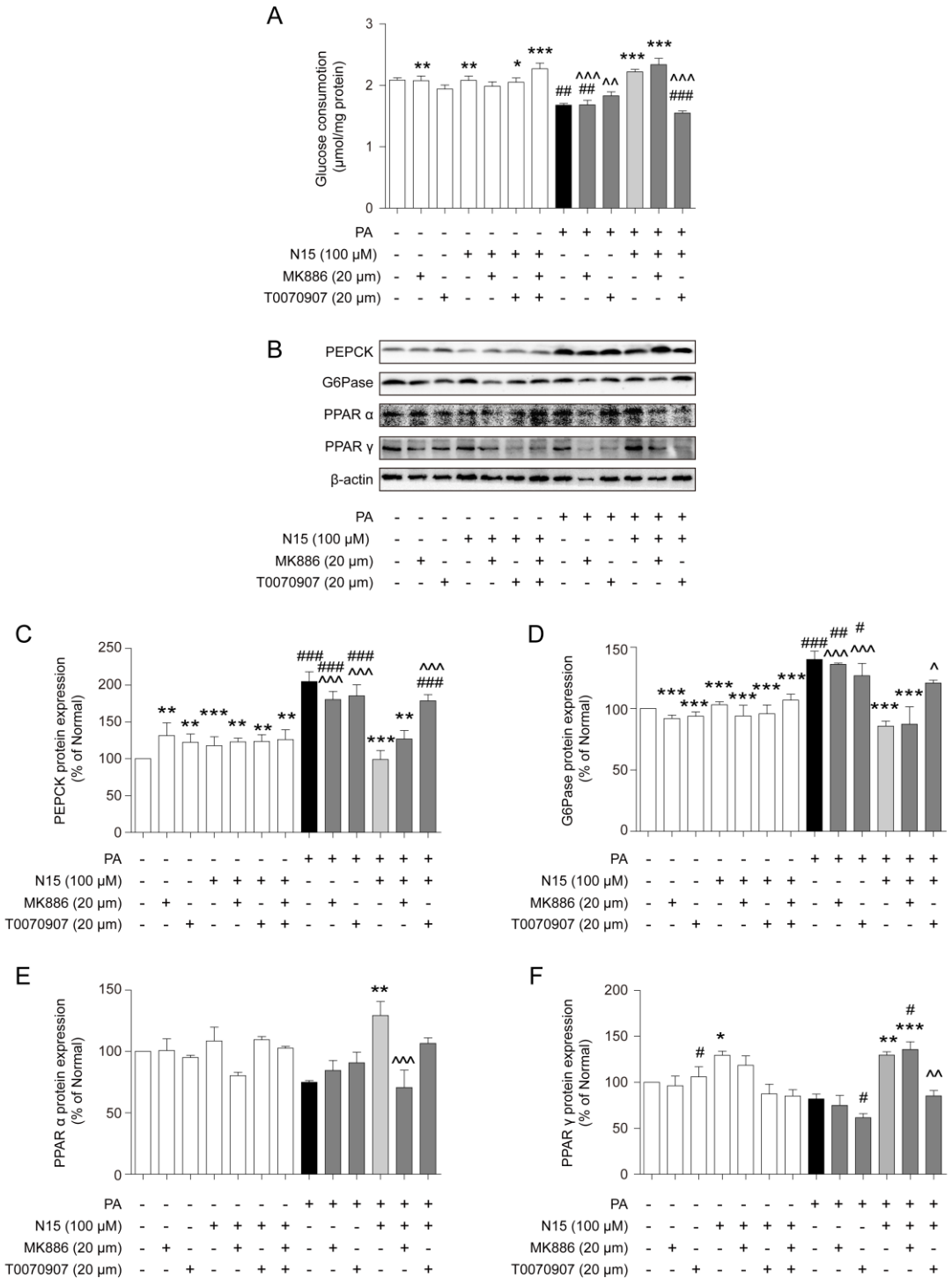
N15 inhibited glucose production may be through PPARγ pathway. (A) The effect of N15 (20, 50 and 100 μM) or pioglitazone (100 μM) on glucose production in PA-stimulated HepG2 cells. (B) Effects of PPARα or PPARγ pathways on the anti-gluconeogenesis action of N15. HepG2 cells were subjected to the PPARα antagonist MK886 (20 μM) or the PPARγ antagonist T0070907 (20 μM) for 2 h before treatment with N15 (100 μM) for an additional 12 h. The level of glucose production was tested in different group. The values are shown as means ± S.E.M of five separate experiments performed in duplicate (n = 5). $^{\#\#\#}$

P < 0.01 vs. normal group; ** P < 0.01, *** P < 0.001 vs. PA group; ^^ P < 0.01, ^^^ P < 0.001 vs. N15-100 group.








A
Glucose consumption (μmol/mg protein)
PA
N15 (100 μM)
MK886 (20 μm)
T0070907 (20 μm)
B
PEPCK
G6Pase
PPAR α
PPAR γ
β-actin
C
PEPCK protein expression (% of Normal)
D
G6Pase protein expression (% of Normal)
E
PPAR α protein expression (% of Normal)
F
PPAR γ protein expression (% of Normal)