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RESEARCH PAPER

# Irbesartan treatment up-regulates hepatic expression of PPARα and its target genes in obese Koletsky ($fa^k/fa^k$) rats: a link to amelioration of hypertriglyceridaemia

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## BACKGROUND AND PURPOSE
Hypertriglyceridaemia is associated with an increased risk of cardiovascular disease. Irbesartan, a well-established angiotensin II type 1 receptor ($AT_1$) blocker, improves hypertriglyceridaemia in rodents and humans but the underlying mechanism of action is unclear.

## EXPERIMENTAL APPROACH
Male obese Koletsky ($fa^k/fa^k$) rats, which exhibit spontaneous hypertension and metabolic abnormalities, received irbesartan (40 mg·kg$^{-1}$·day$^{-1}$) or vehicle by oral gavage over 7 weeks. Adipocyte-derived hormones in plasma were measured by ELISA. Gene expression in liver and other tissues was assessed by real-time PCR and Western immunoblotting.

## KEY RESULTS
In Koletsky ($fa^k/fa^k$) rats irbesartan lowered plasma concentrations of triglycerides and non-esterified fatty acids, and decreased plasma insulin concentrations and the homeostasis model assessment of insulin resistance index. However, this treatment did not affect food intake, body weight, epididymal white adipose tissue weight, adipocyte size and plasma leptin concentrations, although plasma adiponectin was decreased. Irbesartan up-regulated hepatic expression of mRNAs corresponding to peroxisome proliferator-activated receptor (PPAR)α and its target genes (carnitine palmitoyltransferase-1a, acyl-CoA oxidase and fatty acid translocase/CD36) that mediate hepatic fatty acid uptake and oxidation; the increase in hepatic PPARα expression was confirmed at the protein level. In contrast, irbesartan did not affect expression of adipose PPARγ and its downstream genes or hepatic genes that mediate fatty acid synthesis.

## CONCLUSIONS AND IMPLICATIONS
These findings demonstrate that irbesartan treatment up-regulates PPARα and several target genes in liver of obese spontaneously hypertensive Koletsky ($fa^k/fa^k$) rats and offers a novel insight into the lipid-lowering mechanism of irbesartan.

## Abbreviations
ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; AT1, angiotensin II type 1 receptor; ARB, angiotensin II type 1 receptor blocker; CPT, carnitine palmitoyltransferase; DGAT, diacylglycerol acyltransferase; eWAT, epididymal white adipose tissue; FAS, fatty acid synthase; FAT, fatty acid translocase; GLUT, glucose transporter; HOMA-IR, homeostasis model assessment of insulin resistance; NEFA, non-esterified fatty acids; PPAR, peroxisome proliferator-activated receptor; SBP, systolic blood pressure; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein

## Introduction

The metabolic syndrome is a cluster of conditions arising from many factors including genetic mutation, overnutrition and a sedentary lifestyle. Common components of the metabolic syndrome include abdominal obesity, hypertension, dislipidaemia and insulin resistance. Insulin resistance and type 2 diabetes are associated with abnormalities in lipid and lipoprotein homeostasis, including elevated triglycerides, which increase the risk of cardiovascular disease (Krauss, 2004). Hypertriglyceridaemia is considered to be an important risk factor for atherosclerosis and other cardiovascular complications in patients with type 2 diabetes (Ginsberg, 1996), and may also be associated with premature coronary artery disease (Brunzell, 2007). In addition, elevated plasma concentrations of non-esterified fatty acids (NEFA) have been associated with deterioration of glucose tolerance independent of other markers of insulin resistance that characterize subjects who are at risk from type 2 diabetes (Charles *et al.*, 1997). Prolonged elevations of NEFA in plasma can exacerbate the impairment in glucose homeostasis in individuals with obesity and type 2 diabetes (Saloranta and Groop, 1996) and may stimulate gluconeogenesis, the development of insulin resistance in muscle and liver, and may also impair insulin secretion in genetically predisposed individuals (Bergman and Ader, 2000). It has been suggested that NEFAs are a major link between obesity and insulin resistance/type 2 diabetes (McGarry, 2002; Bays *et al.*, 2004). Therefore, pharmacological treatments that decrease circulating triglycerides and NEFAs may improve insulin resistance and reduce the risk of cardiovascular disease.

Irbesartan, one of the earliest angiotensin II type 1 receptor blocker (ARBs) to enter clinical use, is a well-established and widely used antihypertensive agent. Irbesartan has been shown to decrease plasma triglyceride concentrations in the obese Zucker rat (Janiak *et al.*, 2006; Muñoz *et al.*, 2006) and the corpulent JCR:LA-cp rat (Russell *et al.*, 2009). Large-scale clinical trials have also demonstrated that irbesartan improves metabolic parameters, including plasma triglyceride concentrations, in patients with hypertension and the metabolic syndrome (Kintscher *et al.*, 2007; Parhofer *et al.*, 2007). However, the underlying mechanism of these lipid-lowering effects remains unknown.

The genetically obese Koletsky ($fa^k/fa^k$) rat strain carries a nonsense mutation in the leptin receptor gene (Takaya *et al.*, 1996). The $fa^k$ mutation results in hyperphagia, obesity, insulin resistance and hyperlipidaemia (Koletsky, 1973; Koletsky and Ernsberger, 1992; Friedman *et al.*, 1997) superimposed on the background of the spontaneously hypertensive lean Koletsky (+/+) littermates. As ARBs are prescribed to the patients with hypertension, the present study investigated the mechanism of the lipid-lowering effect of irbesartan using the obese spontaneously hypertensive Koletsky ($fa^k/fa^k$) rats. The principal findings to emerge were that, irbesartan decreased plasma triglyceride and NEFA concentrations, in addition to decreases in plasma insulin concentrations and the index of homeostasis model assessment of insulin resistance (HOMA-IR). Peroxisome proliferator-activated receptor (PPAR)α and several PPARα-responsive genes were up-regulated in liver, thus increasing the capacity for uptake and oxidation of fatty acids. In contrast, irbesartan did not significantly affect the expression of PPARγ and downstream genes in white adipose tissues, and the genes responsible for fatty acid synthesis in liver. Thus, irbesartan improves hypertriglyceridaemia and high free fatty acid concentrations via a hepatic PPARα pathway in insulin resistant rats with obesity and hypertension.

## Methods

### *Animals, diet and experimental protocol*

All animal procedures were in accordance with the 'Principles of laboratory animal care' (http://grants1.nih.gov / grants / olaw / references / phspol.htm) and were approved by the Animal Ethics Committee, Kyoto University, Japan.

Male obese Koletsky ($fa^k/fa^k$) rats and their lean (+/+) littermates aged 10–11 weeks were generous gifts from Japan SLC, Inc., Shizuoka, Japan. Rats were housed in a temperature-controlled facility (21 ± 1°C, 55 ± 5% relative humidity) with a 12-h light/dark cycle (2 rats per cage). Animals were allowed free access to water and the standard diet (CLEA Tokyo, Japan) for 1 week before starting the experiments. Rats were divided into three groups ($n$ = 6 per group): lean control (+/+ Irb -), obese control ($fa^k/fa^k$ Irb -) and obese with irbesartan treatment ($fa^k/fa^k$ Irb +). There was no difference in body weight between two obese groups before treatments. Animals in the $fa^k/fa^k$ Irb + group were administered irbesartan (nomenclature follows Alexander *et al.*, 2008) (40 mg·kg$^{-1}$, a generous gift from Shionogi & Co., Ltd, Japan, suspended in 5% Gum Arabic) by oral gavage once daily (11 h 00 min–12 h 00 min) for 7 weeks. The rats in the +/+ Irb – and $fa^k/fa^k$ Irb – groups received vehicle (5% gum arabic) alone. The rats were weighed once every 3–4 days to determine gavage volume and daily food intake was estimated from weekly measurements. Systolic blood pressure

(SBP) was measured at Week 1. Blood samples were collected by retro-orbital venous puncture under ether anaesthesia at Week 5 in animals that had been deprived of food for 6 h, for determination of plasma concentrations of triglyceride and NEFA using enzymatic methods (kits from Wako, Osaka, Japan), leptin (Morinaga, Tokyo, Japan) and adiponectin (Otsuka Pharmaceutical, Tokushima, Japan) using commercial ELISAs. Plasma glucose and insulin concentrations were determined using enzymatic (kit from Wako, Osaka, Japan) and ELISA (kit from Morinaga, Tokyo, Japan) methods, respectively, at Week 6 after the rats had been deprived of food for 12 h. The HOMA-IR index was calculated as an indicator of insulin sensitivity according to the following formula: [insulin ($\mu IU \cdot mL^{-1}$) × glucose (mM)]/22.5. Animals were weighed at Week 7 and then killed by prompt dislocation of the neck vertebra. Epididymal white adipose tissue (eWAT) and liver were collected and weighed. The gastrocnemius muscle [contains red (mostly type IIa muscle fibres) and white (primarily type IIb fibres) skeletal muscle] was also collected. Segments of each of eWAT, liver and skeletal muscle were snap frozen in liquid nitrogen and stored at –80°C for subsequent determination of triglyceride content and/or gene analysis.

### *SBP*

Systolic blood pressure was measured (2–5 h after administration of irbesartan or vehicle) in conscious rats by a tail-cuff method (MK-2000ST; Muromachi Kikai Co Ltd, Tokyo, Japan). At least six readings were taken for each measurement.

### *Determination of triglyceride content in liver and skeletal muscle*

Triglyceride contents in liver and skeletal muscle were determined as described previously (Oakes *et al.*, 2001). Briefly, 100 mg of tissue was homogenized and extracted with 2 mL of isopropanol. After centrifugation (1000× *g*), the triglyceride content in the supernatants was determined enzymatically (Wako, Osaka, Japan).

### *Histological examination*

A portion of eWAT or liver was fixed with 10% formalin and embedded in paraffin. Four-micron sections were cut and stained with haematoxylin and eosin for examination of adipose tissue and liver histology (IX-81, Olympus Corporation, Tokyo, Japan). The adipocyte cross-sectional area was measured using an image analysing system (KS 400 Imaging System; Carl Zeiss Vision, Eching, Germany).

### *Gene expression analysis*

Total RNA was isolated from the eWAT, livers and skeletal muscle of individual mice using TRIzol (Invitrogen, Osaka, Japan). Single-stranded cDNA was synthesized from 1 μg of total RNA using SuperScript III First-Strand Synthesis System for RT-PCR, according to the manufacturer's instructions (Invitrogen, Osaka, Japan). Quantitative real-time PCR was performed with an AB 7300 Real-Time PCR System using TaqMan (Applied Biosystems, USA). The sequences of primers and probes (Sigma-Genosys, Japan) used in the present study are shown in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as an endogenous control (housekeeping gene).

Protein expression was quantified by Western blotting (Lorenzo *et al.*, 2002). Tissue proteins were resolved on 4–12% polyacrylamide gels in the presence of sodium dodecylsulphate, transferred electrophoretically to polyvinylidene difluoride membranes, blocked (in buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 5% bovine serum albumin, 0.1% Tween-20) and incubated at 4°C for 18 h with PPARα-specific antibody (1:300; Santa Cruz, CA, USA). Detection was performed with peroxidase-conjugated secondary antibody, by enhanced chemiluminescence (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Immunoblotting with a monoclonal anti-β-actin antibody (Cell Signaling, Beverly, MA, USA) was conducted to ensure equal protein loading.

### *Data analysis*

All results are expressed as means ± SEM. Data obtained from experiments with three groups of animals (Figures 1–4) were analysed by one-way analysis of variance (ANOVA). If a difference was detected (*F*-ratio), the Student-Newman-Keuls test was performed to locate the differences between groups. Data obtained from experiments with two groups of animals (Figure 5) were analysed by Student's *t*-test; $P < 0.05$ was considered to be statistically significant.

## Results

### *Metabolic abnormalities and effects of irbesartan in obese Koletsky ($fa^k/fa^k$) rats*

Animals were genotyped by the supplier. Obese Koletsky ($fa^k/fa^k$) rats appeared to be somewhat larger than their lean littermates; rats of both genotype were hypertensive (SBP: ≈170 mmHg; Figure 1A) compared with normal controls (SBP: ≈120 mmHg). Food intake (Figure 1C) and body

## Table 1

Primer and probe sequences for real time RT-PCR assays

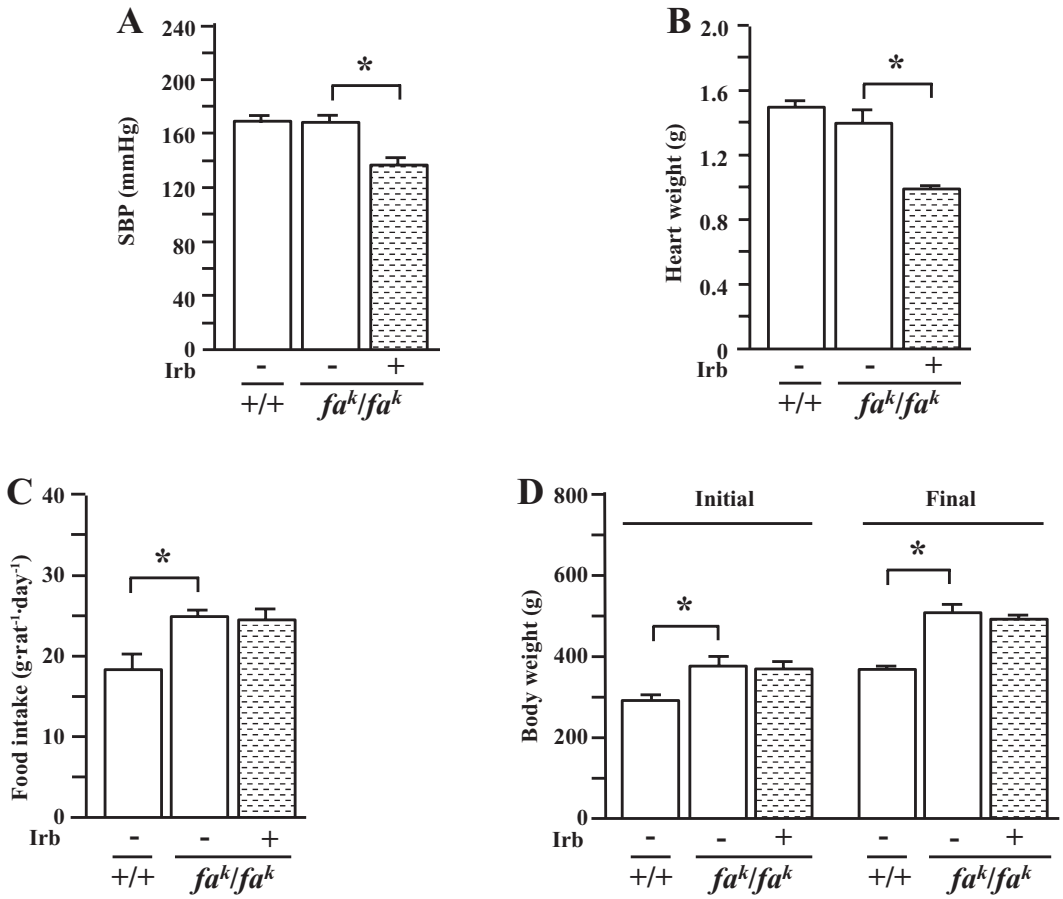
| Gene | Probe | Primers[a] |
|---|---|---|
| GAPDH | TTGTGCAGTGCCAGCCTCGTCTCA | *f* TGTTCTAGAGACAGCCGCATCTT |
| | | *r* CCGACCTTCACCATCTTGTCTAT |
| PPARγ | CCTGCGGAAGCCCTTTGGTGACT | *f* TGACCAGGGAGTTCCTCAAAA |
| | | *r* AGCAAACTCAAACTTAGGCTCCAT |
| FAS | ACCATCTCTGGACCTCAGGCTGCAGT | *f* TGCCTGCCTGCCACAAC |
| | | *r* CTTGCTTTAGCTGCTCCACAAATT |
| ACC1 | CAGCACAGCTCCAGATTGCCATGG | *f* GGTGGCTGATGTCAATCTTCCT |
| | | *r* TCATACGAATATCCTTGATCCTAAATAGAG |
| CD36 | CTTGGATGTGGAACCCATAACTGGATTC | *f* CCTAACGAAGATGAGCATAGGACAT |
| | | *r* GTTGACCTGCAGTCGTTTTGC |
| SCD1 | CCGGGCCCATTCATACACATCGTTCT | *f* CCTCATCATTGCCAACACCAT |
| | | *r* GGCGTGTGTCTCAGAGAACTTG |
| SREBP1c | CAAAGCTGAATAAATCTGCTGTCTTGCGCA | *f* CCTGGTGGTGGGCACTGA |
| | | *r* GTGCTGTAAGAAGCGGATGTAGTC |
| PPARα | CTGCAAGGGCTTCTTTCGGCGA | *f* CTATGGAGTCCACGCATGTGAA |
| | | *r* TTGTCGTACGCCAGCTTTAGC |
| CPT1a | CCCCGCGAATCCGTCCAGC | *f* GGTTCAAGAATGGCATCATCACT |
| | | *r* TCACACCCACCACCACGATA |
| ACO | CAGACGGAGATGGGCCACGGAAC | *f* AAGAACTCCAGATAATTGGCACCTA |
| | | *r* TGGTTTCCAAGCCTCGAAGAT |
| CPT1b | CGAGCAGTGCCAGACAGCCATCG | *f* CGGATGCAGTGGGACATTC |
| | | *r* CCAGGGCCTTGGCTACTTG |
| aP2 | TGGGAGTTGGCTTCGCCACCAG | *f* TCCAGTGAGAACTTCGATGATTACA |
| | | *r* GGCCATACCGGCCACTTT |
| Adiponectin | TTCTCTCCAGGAGTGCCATCTCTGCC | *f* GGACCAAGAACACCTGCGTCT |
| | | *r* TCCTGGTCACAATGGGATACC |
| DGAT1 | CAGAACTCCATGAAGCCCTTCAAGGACAT | *f* CAGCAGTGGATGGTCCCTACTAT |
| | | *r* AAGAGACGCTCAATGATTCGTG |
| GLUT4 | CATCAACGCCCCACAGAAAGTGATTG | *f* GCTCCCTTCAGTTTGGCTATAACA |
| | | *r* GCCAAGTTGCATTGTAGCTCTGT |

[a]Forward primers are designated by *f* and reverse primers by *r*.
Sequences: 5′ to 3′.
GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; CPT, carnitine palmitoyltransferase; DGAT, diacylglycerol acyltransferase; FAS, fatty acid synthase; GLUT, glucose transporter; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein.

weights (Figure 1D) were greater in obese rats than in lean controls, but there was no difference in heart weight between the genotypes (Figure 1B). Irbesartan treatment (40 mg·kg$^{-1}$) decreased SBP by ~40 mmHg (Figure 1A) and heart weight (Figure 1B) in obese rats, consistent with its cardiovascular actions. However, this treatment did not significantly affect food intake (Figure 1C) and body weight (Figure 1D).

Compared with lean controls, plasma triglyceride concentrations were higher in obese Koletsky ($fa^k/fa^k$) rats under both fasting (deprived of food) and non-fasting conditions (Figure 2A). Plasma NEFA concentrations were also higher in obese rats under fasting, but not non-fasting, conditions (Figure 2B). Treatment of obese rats with irbesartan for 5 weeks decreased both fasted and non-fasted plasma triglyceride and fasted NEFA concentrations, but it was without effect on non-fasted NEFA concentrations in plasma of obese rats (Figure 2A and B).

In fasted animals, plasma glucose concentrations did not differ between rats of either genotype (Figure 2C). However, plasma insulin concentra-




## Figure 1

Systolic blood pressure (SBP) (A), heart weight (B), food intake (C) and initial and final body weight (D) in male lean (+/+) and obese ($fa^k/fa^k$) Koletsky rats. Animals received irbesartan (Irb) (40 mg·kg$^{-1}$·day$^{-1}$) or vehicle by oral gavage for 7 weeks. SBP was measured with a tail-cuff method at Week 1 after treatment. Food intake over 24 h was determined at Week 3. All values are means ± SEM ($n = 6$ each group). Irb -: vehicle; Irb +: irbesartan; *$P < 0.05$.

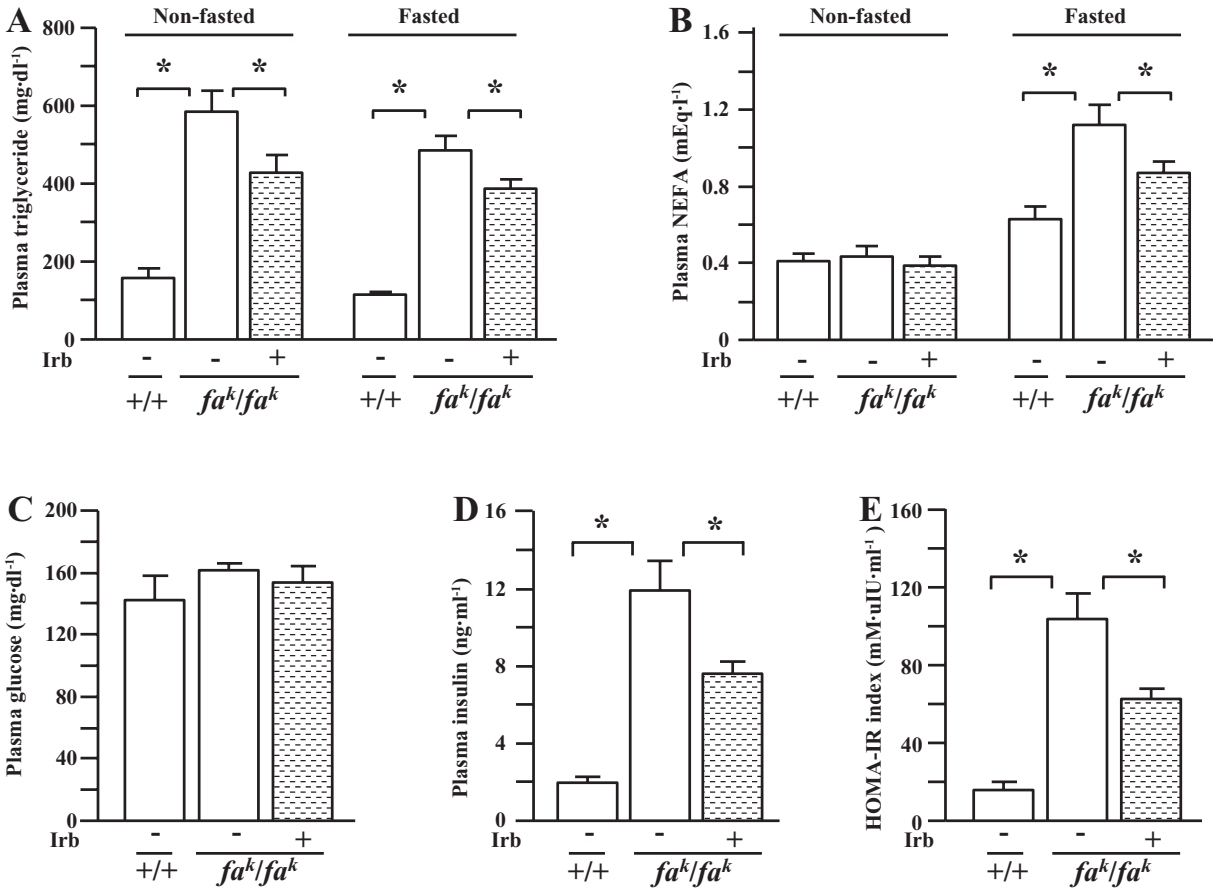
tions (Figure 2D) and the HOMA-IR index (Figure 2E) were considerably higher in the obese rats than in their lean counterparts. Although irbesartan treatment was without effect on plasma glucose concentration, plasma insulin concentrations and the HOMA-IR index in obese rats were decreased by this treatment (Figure 2D and E).

Hepatomegaly (Figure 3A) was evident in the obese Koletsky ($fa^k/fa^k$) rat; consistent with this finding, hepatic triglyceride content was increased markedly in these animals (Figure 3B and C), compared with corresponding lean controls. Increased vacuolization was evident on histological examination of liver sections from obese rats (Figure 3F) compared with lean rats (Figure 3E), indicative of excess lipid droplet accumulation. In skeletal muscle, triglyceride content was also increased in obese rats (Figure 3D); irbesartan treatment did not significantly alter these parameters (Figure 3A–D and G).

Compared with lean control rats, eWAT weight (Figure 4A), adipocyte size (Figure 4B and F) and plasma leptin concentrations (Figure 4C) were greater in obese Koletsky rats, but plasma adiponectin concentrations (Figure 4D) were not different between lean and obese animals. In obese rats irbesartan decreased plasma adiponectin concentrations (Figure 4D) but did not affect eWAT weight (Figure 4A), adipocyte size (Figure 4B and G) and plasma leptin concentrations (Figure 4C).

### *Gene expression profile in obese Koletsky ($fa^k/fa^k$) rats*

By real-time PCR obese Koletsky ($fa^k/fa^k$) rats showed a significant increase in hepatic and adipose, but not muscular, expression of GAPDH, compared with lean rats; irbesartan treatment did not significantly affect GAPDH expression (data not shown). Thus, comparisons in gene expression were restricted




## Figure 2

Non-fasted and fasted (rats deprived of food) plasma concentrations of triglyceride (A) and non-esterified fatty acids (NEFA) (B), fasted plasma glucose (C) and insulin (D) concentrations, and the index of the homeostasis model assessment of insulin resistance (HOMA-IR) (E) in male lean (+/+) and obese ($fa^k/fa^k$) Koletsky rats; animals received irbesartan (Irb) or vehicle as described in the legend to Figure 1. All values are means ± SEM ($n = 6$ each group). Irb -: vehicle; Irb +: irbesartan; *$P < 0.05$.

to obese animals, with or without irbesartan treatment.

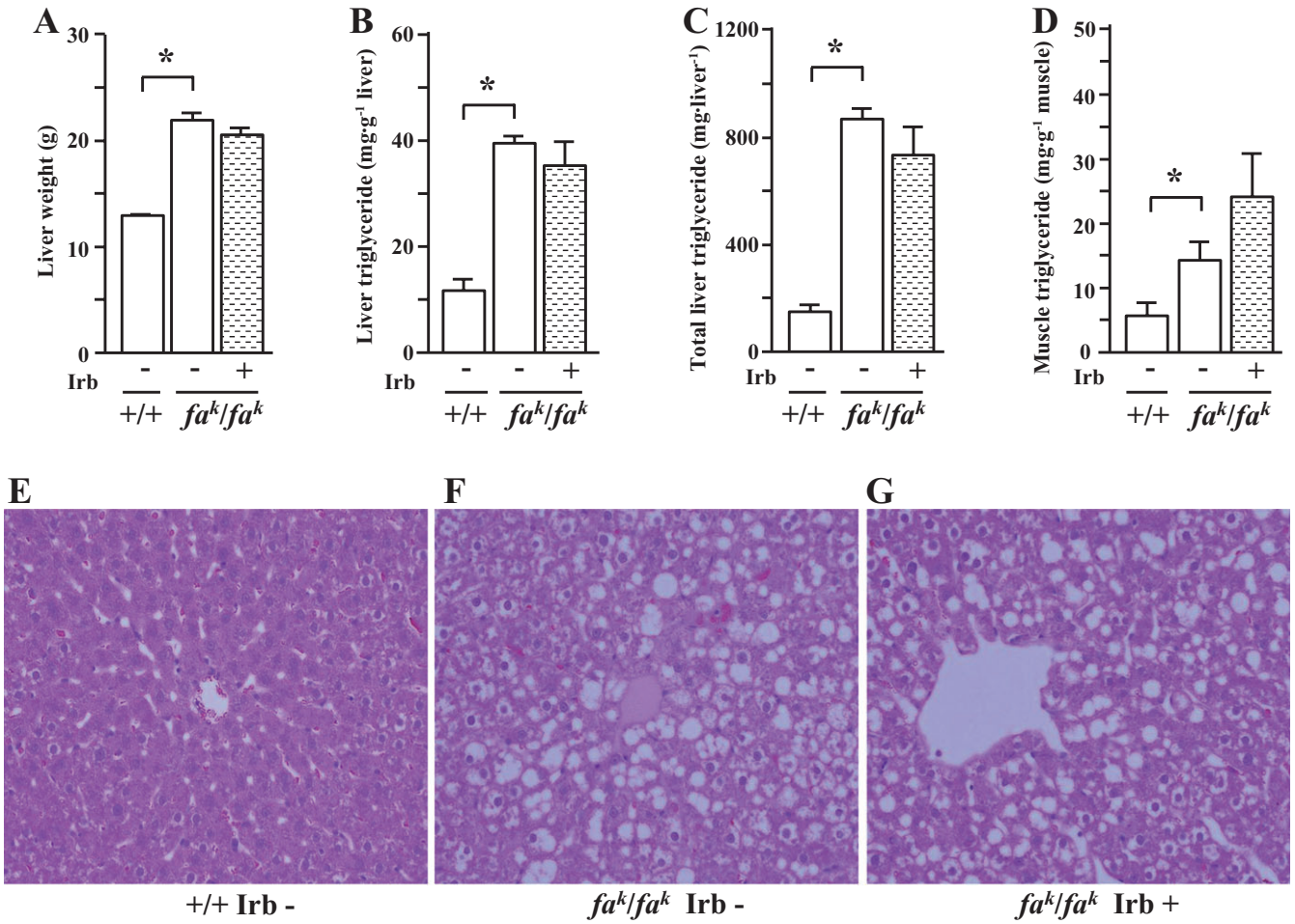
Interestingly, irbesartan treatment up-regulated PPARα, carnitine palmitoyltransferase (CPT)1a, acyl-CoA oxidase (ACO) and fatty acid translocase (FAT)/CD36 (Figure 5A) mRNAs in liver of obese Koletsky ($fa^k/fa^k$) rats; the increase in PPARα expression was confirmed at the protein level by Western immunoblotting (Figure 5D). However, irbesartan treatment did not alter hepatic sterol regulatory element-binding protein (SREBP)1c, acetyl-CoA carboxylase (ACC)1, fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD)1 mRNA expression, but increased the level of diacylglycerol acyltransferase (DGAT)1 mRNA (Figure 5A).

In contrast with these findings in liver, irbesartan treatment did not alter the expression of PPARα, CPT1a, ACO and FAT/CD36 mRNAs in skeletal muscle of obese Koletsky ($fa^k/fa^k$) rats (data not shown). Consistent with decreased HOMA-IR index, irbesartan up-regulated muscular glucose transporter (GLUT)4 expression (Figure 5B).

In white adipose tissue from obese Koletsky ($fa^k/fa^k$) rats, irbesartan treatment did not significantly change the expression of mRNAs encoding PPAR&gamma, aP2, adiponectin, FAS, ACC1, CD36, SCD1, SREBP1c and DGAT1 (data not shown). However, GLUT4 mRNA level was increased by irbesartan treatment (Figure 5C).

## Discussion

Consistent with recent clinical findings (Kintscher *et al.*, 2007; Parhofer *et al.*, 2007), the present study demonstrates that irbesartan treatment improves hypertriglyceridaemia and reduces free fatty acid concentrations in obese Koletsky ($fa^k/fa^k$) rats that exhibit hypertension and metabolic abnormalities.

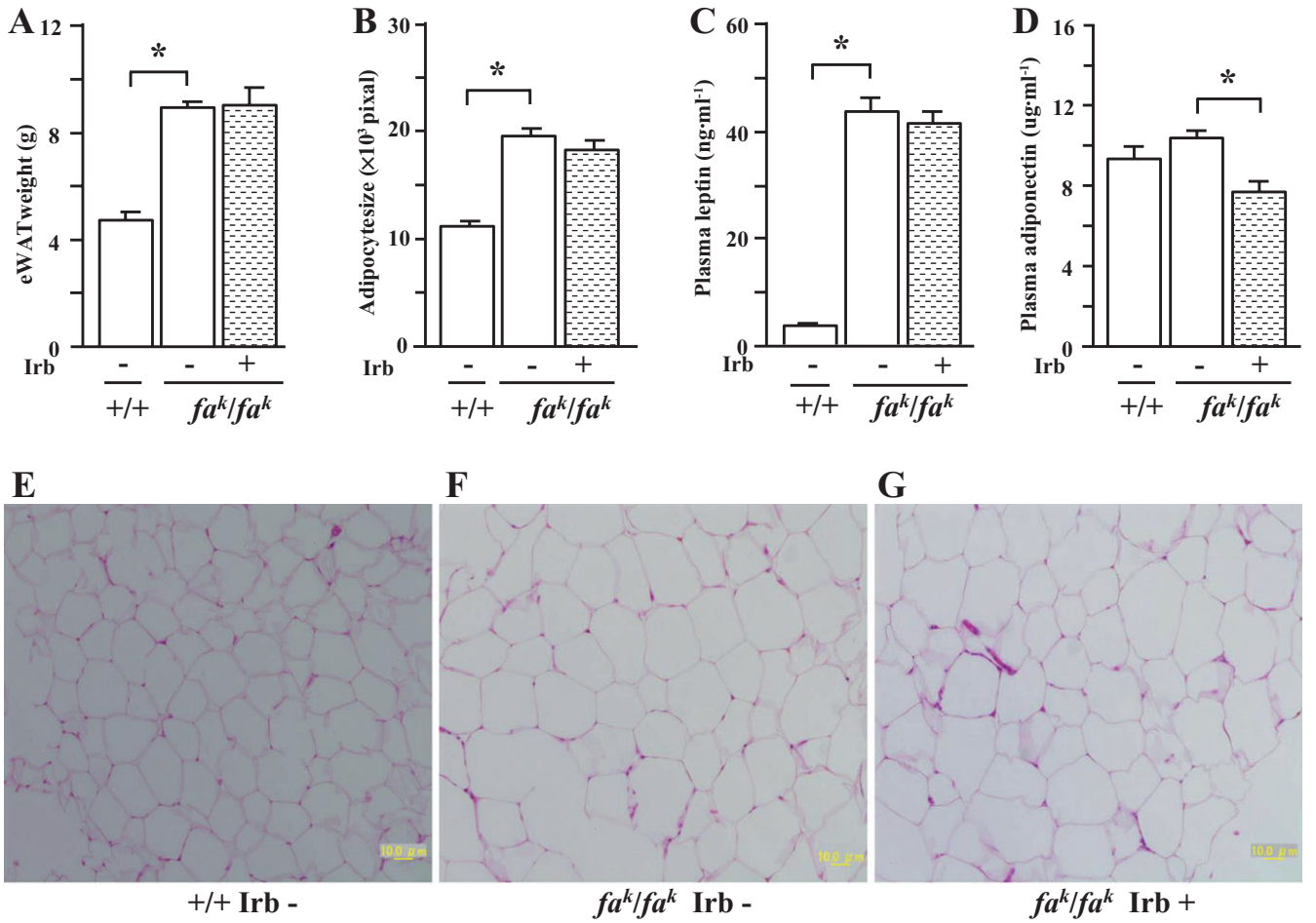



## Figure 3

Liver weight (A), triglyceride contents in liver (B, C) and skeletal muscle (D), and representative images showing histology of liver (Hematoxylin and eosin–staining, X200) (E-G) in male lean (+/+) and obese ($fa^k/fa^k$) Koletsky rats; animals received irbesartan (Irb) or vehicle as described in the legend to Figure 1. All values are means ± SEM ($n = 6$ each group). Irb -: vehicle; Irb +: irbesartan; *$P < 0.05$.

Improvements in plasma lipid concentrations are not produced by all ARBs. Telmisartan, valsartan, candesartan, olmesartan and losartan were without effect on serum triglyceride concentrations in hypertensive patients with metabolic syndrome and/or type 2 diabetes (Bahadir *et al.*, 2007; Ichikawa, 2007; Tomiyama *et al.*, 2007; Nakayama *et al.*, 2008). Genetic blockade of $AT_1$ also failed to decrease serum triglyceride concentrations in mice fed high fat (Kouyama *et al.*, 2005) or methionine-choline deficient diets (Nabeshima *et al.*, 2009). Similarly, telmisartan and valsartan treatment did not alter serum triglyceride concentrations in rats fed a diet that was high in fat and carbohydrate (Sugimoto *et al.*, 2006). Losartan (Xu *et al.*, 2005) and olmesartan (Yokozawa *et al.*, 2009) did not significantly affect serum triglyceride and/or NEFA concentrations in obese Zucker rats. Thus, it appears that mechanisms other than $AT_1$ inhibition may mediate the lipid-lowering actions of irbesartan.

peroxisome proliferator-activated receptor α is a member of the nuclear receptor superfamily. PPARα is predominantly expressed in liver and, to a lesser extent, in skeletal muscle and heart, where it has a crucial role in controlling fatty acid oxidation (Reddy and Hashimoto, 2001). Fibrates are established pharmacological activators of PPARα that decreases circulating triglycerides and improves insulin sensitivity. Interestingly, in the present study irbesartan treatment was found to up-regulate PPARα and several target genes that mediate fatty acid oxidation in liver of obese Koletsky ($fa^k/fa^k$) rats. Furthermore, hepatic expression of FAT/CD36, another PPARα target gene that is important in facilitating cellular fatty acid uptake and ameliorating insulin resistance in rodents and humans (Pravenec *et al.*, 2001; Su and Abumrad, 2009), was also up-regulated by irbesartan. However, irbesartan did not significantly enhance the expression of these genes in skeletal muscle. Irbesartan also did

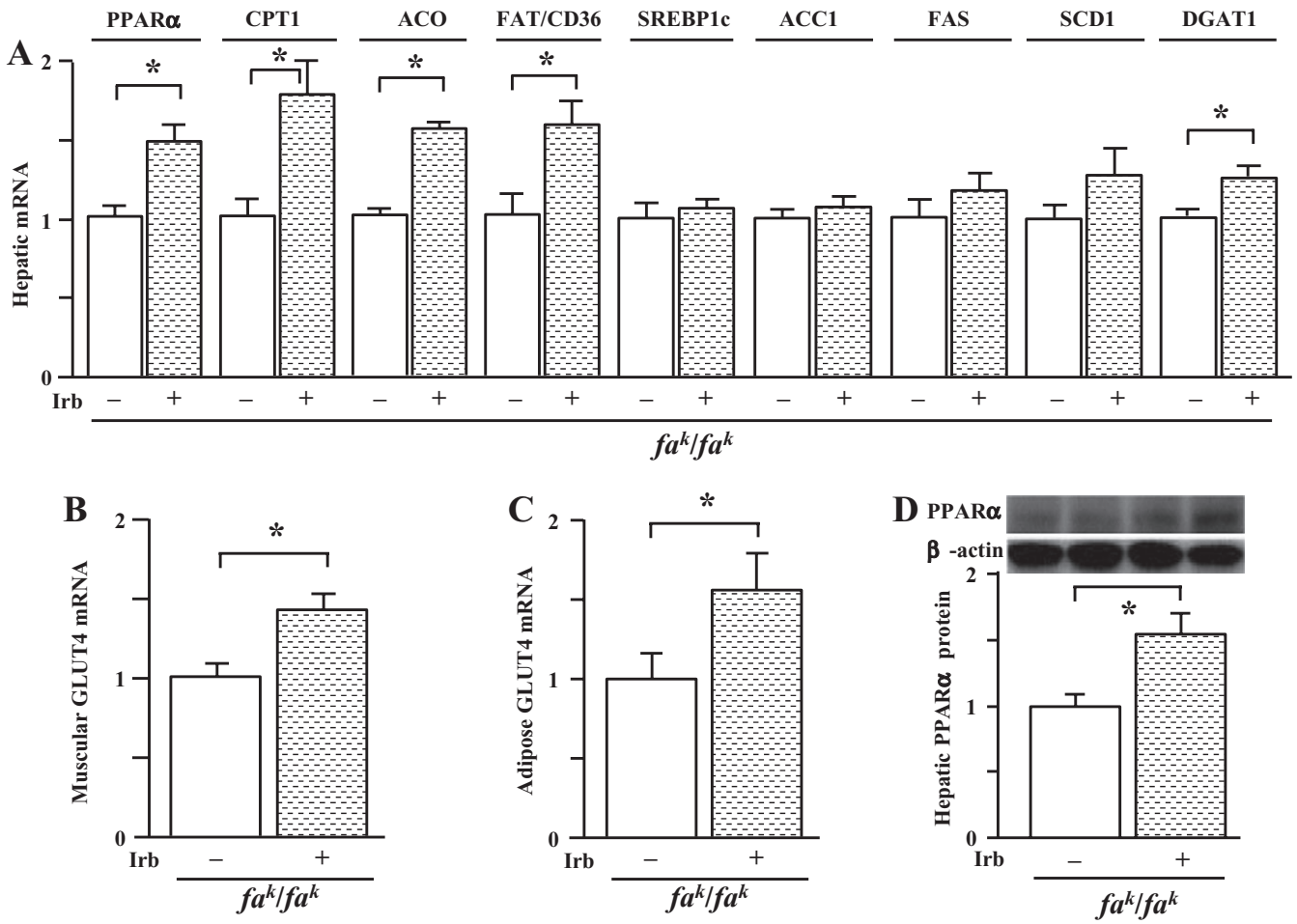



## Figure 4

Epididymal white adipose tissue (eWAT) weight (A), adipocyte size (B), fasted (rats deprived of food) plasma concentrations of leptin (C) and adiponectin (D), and representative images showing histology of eWAT (haematoxylin and eosin–staining, X200) (E-G) in male lean (+/+) and obese ($fa^k/fa^k$) Koletsky rats; animals received irbesartan (Irb) or vehicle as described in the legend to Figure 1. All values are means ± SEM ($n$ = 6 each group). Irb -: vehicle; Irb +: irbesartan; *$P < 0.05$.

not affect the hepatic expression of the genes that mediate fatty acid synthesis, including SREBP1c, FAS, ACC1 and SCD1, in obese Koletsky ($fa^k/fa^k$) rats. Thus, the present findings suggest that the actions of irbesartan in increasing fatty acid uptake and oxidation by the liver are mediated by a hepatic PPARα pathway and lead to decreased plasma triglyceride and free fatty acid concentrations. However, irbesartan also increased hepatic expression of DGAT1, a key enzyme in triglyceride synthesis (Villanueva *et al.*, 2009). It is possible that the unchanged hepatic lipid content after irbesartan treatment may reflect minimal overall effects on the balance between fatty acid uptake, β-oxidation, esterification and lipid secretion/storage.

In contrast to the situation with PPARα, PPARγ is expressed predominantly in adipose tissue and at only low levels in liver and skeletal muscle. PPARγ-activating ligands improve adipose tissue function by altering fat topography and adipocyte phenotype and by up-regulating genes involved in fatty acid metabolism and triglyceride storage (Sharma and Staels, 2007). Furthermore, PPARγ activation is associated with potentially beneficial effects on the secretion of a range of factors from adipose tissue, including adiponectin, thereby improving insulin sensitivity (Sharma and Staels, 2007). Indeed, adiponectin is an important mediator of the improvement in insulin sensitivity elicited by PPARγ agonists (Sharma and Staels, 2007). The increase in plasma adiponectin concentrations observed after thiazolidinedione therapy is closely associated with a decline in hepatic fat content (Sharma and Staels, 2007). Thus, treatment with rosiglitazone enhances insulin sensitivity, and is accompanied by decreased plasma triglyceride and NEFA concentrations, increased plasma adiponectin and leptin concentrations, and increased eWAT weight in mice fed high




## Figure 5

Gene expression profile. (A) mRNAs encoding peroxisome proliferator-activated receptor (PPAR)α, carnitine palmitoyltransferase (CPT)1a, acyl-CoA oxidase (ACO), fatty acid translocase (FAT)/CD36, sterol regulatory element-binding protein (SREBP)1c, acetyl-CoA carboxylase (ACC)1, fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD)1 and diacylglycerol acyltransferase (DGAT)1 (B) GLUT4 mRNA in skeletal muscle (C) GLUT4 mRNA in eWAT and (D) protein expression of PPARα in liver of male obese Koletsky ($fa^k/fa^k$) rats that were either untreated (-) or treated (+) with irbesartan (Irb) as described in Figure 1. Total RNA was isolated from liver, skeletal muscle or eWAT of individual rats using TRIzol. Quantitative PCR results were normalized to GAPDH, while the results from Western blot analysis were normalized to β-actin. Levels in obese control rats were arbitrarily assigned a value of 1. All values are means ± SEM ($n = 6$ each group). *$P < 0.05$. eWAT, epididymal white adipose tissue; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter.

fat-containing diets (Stienstra *et al.*, 2008; Kuda *et al.*, 2009). Rosiglitazone treatment also decreased plasma glucose and triglyceride concentrations, but increased plasma adiponectin and leptin concentrations, as well as body weight in obese Zucker rats (Cai *et al.*, 2000; Reifel-Miller *et al.*, 2005). In addition, rosiglitazone stimulated adipose activity of DGAT, a key enzyme catalysing triglyceride synthesis, which is accompanied by a specific increase in the mRNA corresponding to adipose DGAT1, but not DGAT2, in rats (Festuccia *et al.*, 2009). Irbesartan has been shown to enhance PPARγ-dependent 3T3-L1 adipocyte differentiation, as reflected by increases in expression of certain adipogenic marker genes (Benson *et al.*, 2004; Schupp *et al.*, 2004). However, findings from immunohistochemical studies indicated that irbesartan treatment decreased PPARγ expression in the white and brown adipose tissues of obese Zucker rats in a dose-dependent fashion (Di Filippo *et al.*, 2005). In the present study, treatment of obese Koletsky ($fa^k/fa^k$) rats with irbesartan decreased plasma adiponectin concentrations, but was without effect on eWAT weight, adipocyte size and plasma leptin concentrations. In adipose tissue of Koletsky ($fa^k/fa^k$) rats, irbesartan was without effect on expression of PPARγ and its downstream target genes aP2, adiponectin, FAS, ACC1, CD36, SCD1, SREBP1c and DGAT1. Thus, our present findings do not support the contention that irbesartan improves hyperlipidaemia

and insulin sensitivity by modulating PPARγ signalling in adipose tissue of obese Koletsky rats. Further investigations are required to evaluate whether irbesartan also modulates expression of PPARβ/&delta, the other PPAR isoform involving lipid metabolism.

Muñoz *et al*. reported that irbesartan (50 mg·kg$^{-1}$ for 6 months) decreased lipid accumulation in the liver of obese Zucker rats (Muñoz *et al*., 2006; Toblli *et al*., 2008). This group also demonstrated that irbesartan reduced adipocyte size and increased adiponectin expression in eWAT from these animals (Muñoz *et al*., 2009). However, the present findings demonstrated that irbesartan (40 mg·kg$^{-1}$ for 7 weeks) did not significantly affect hepatic steatosis, eWAT weight, plasma leptin concentration, adipocyte size or adipose adiponectin gene expression in obese Koletsky ($fa^k/fa^k$) rats. Further, both Russell *et al*. (2009), using the insulin-resistant JCR:LA-cp rat and the present study found that irbesartan treatment decreased plasma adiponectin concentrations. There are several possible factors that may be responsible for these discrepancies. First, the animal strains are different: although both obese Zucker rats and obese Koletsky rats carry leptin receptor mutations, there are still some important differences. In the fatty Zucker rat the $Lepr^{fa}$ gene carries a point mutation in codon 269 that produces an amino acid sequence change adjacent to the ligand-binding domain of the receptor (Chua *et al*., 1996); these animals are still responsive to leptin (Cusin *et al*., 1996; Wang *et al*., 1998; Wildman *et al*., 2000). In contrast, the Koletsky rat ($Lepr^{fak}$) carries a premature stop codon and the mutant receptor lacks a transmembrane domain. This truncates all known isoforms of the receptor and, unlike the $Lepr^{fa}$ mutation, the $Lepr^{fak}$ mutation is null (Takaya *et al*., 1996; Wu-Peng *et al*., 1997; Wildman *et al*., 2000). Second, blood pressure differs between the two rat strains: young obese Zucker rats (at least until 18 weeks of age) are normotensive (Muñoz *et al*., 2006; Toblli *et al*., 2008). In contrast, obese Koletsky rats are spontaneously hypertensive (SBP ≈ 170 mmHg at 10 weeks of age); which remained in excess of 130 mmHg after irbesartan treatment (Figure 1A). Finally, the different doses of irbesartan (50 mg·kg$^{-1}$ vs. 40 mg·kg$^{-1}$) and durations of treatments (6 months vs. 7 weeks) were used in the Zucker and Koletsky rats so that comparisons are not straightforward.

In the present study, irbesartan treatment also decreased plasma insulin concentration and the HOMA-IR index in obese Koletsky rats. The results of quantitative PCR analysis demonstrated that GLUT4 gene expression in adipose tissue and skeletal muscle was increased by irbesartan treatment. These results suggest that insulin sensitivity improves after irbesartan treatment. NEFAs are a major link between obesity and insulin resistance/type 2 diabetes (McGarry, 2002; Bays *et al*., 2004). Although a decrease in plasma NEFAs by irbesartan treatment may be associated with enhanced insulin sensitivity, the underlying molecular mechanisms require further investigation. It has been demonstrated that angiotensin II decreases local blood flow both in adipose and skeletal muscle tissue of normal-weight and obese subjects (Goossens *et al*., 2004). Increased muscular blood flow is associated with increased glucose utilization (Baron *et al*., 1992; Wiernsperger, 1994). Further specific investigations are required to determine the involvement of this increased blood flow in the enhanced insulin sensitivity induced by irbesartan

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## Conflicts of interest

None.

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