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The IRS/PI3K/Akt signaling pathway mediates olanzapine-induced hepatic insulin resistance in male rats

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ABSTRACT

Aims: Chronic treatment with antipsychotics, especially most of atypical ones, leads to development of metabolic abnormalities. Olanzapine is an atypical antipsychotic widely used in the treatment of schizophrenia and bipolar disorder. The mechanisms underlying olanzapine-induced metabolic adverse effects in the liver, however, remain unclear. This study was designed to investigate olanzapine-induced insulin-desensitivity in the liver.

Main methods: Male rats were treated with olanzapine (5 mg/kg, by a gavage method, once daily for consecutive 8 weeks. Blood and liver variables were determined enzymatically or histologically. Gene/protein expression was analyzed by real-time PCR and Western blot.

Key findings: Olanzapine treatment significantly increased fasting plasma insulin concentration, the index of the homeostasis model assessment of insulin resistance (HOMA-IR), and hepatic triglyceride and fatty droplet accumulation in rats. Hepatic gene/protein expression profile revealed that olanzapine activated mRNA and protein expression of sterol regulatory element-binding protein-1c, and mRNA levels of its downstream lipogenic enzymes, acetyl-CoA carboxylase-1, fatty acid synthase and stearoyl-CoA desaturase-1. More importantly, phosphorylated protein level of both Ser^{307} in insulin receptor substrate (IRS)-1 and Ser^{731} in IRS-2 was increased. Furthermore, phosphorylation of Tyr^{607} in phosphoinositide 3-kinase (PI3K) p85α, Ser^{473} in Akt and Ser^{2448} in mammalian target of rapamycin was also enhanced.

Significance: Our results suggest that the IRS/PI3K/Akt signaling pathway mediates olanzapine-induced hepatic insulin resistance in male rats. Our findings may provide better understanding of the antipsychotic-induced metabolic adverse effects.

1. Introduction

Insulin is a well-known hormone that is associated with regulation of glucose and lipid metabolism. When insulin receptors in the insulin target tissues fail to respond appropriately to insulin, insulin resistance happens. The statement is existent in a majority of people with the metabolic syndrome. Insulin resistance is associated with various modern chronic illnesses, such as dyslipidemia, type 2 diabetes, non-alcoholic fatty liver diseases and cardiovascular diseases [1]. Recently, increasing worldwide prevalence of neuropsychiatric disorders results in dramatically-expanded use of antipsychotics [2,3]. Antipsychotics are known to link to many metabolic changes. Chronic treatment with antipsychotic drugs, especially most of atypical ones, leads to development of metabolic abnormalities, such as insulin resistance, hypertriglyceridemia and fatty liver in rodents and humans [2–4]. Olanzapine is an atypical antipsychotic that is widely used in the treatment of schizophrenia and bipolar disorder. Although this second-generation antipsychotic has a superior effect on schizophrenia and a lower risk of causing movement adverse effects than first-generation antipsychotics, it often induces more metabolic side effects [3]. Many studies have

Abbreviations: ACC, acetyl-CoA carboxylase; ChREBP, carbohydrate-response element-binding protein; tAkt, total Akt; $pSer^{473}$, phosphorylated Ser^{473} in Akt; FAS, fatty acid synthase; HOMA-IR, the homeostasis model assessment of insulin resistance; IRS, insulin receptor substrate; tIRS, total insulin receptor substrate; $pSer^{2448}$, phosphorylated Ser^{2448} in mammalian target of rapamycin (mTOR); $pSer^{307}$, phosphorylated Ser^{307} in IRS-1; $pSer^{731}$, phosphorylated Ser^{731} in IRS-2; NEFA, non-esterified fatty acid; PI3K, phosphoinositide 3-kinase; tPI3K, total phosphoinositide 3-kinase; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein; $pTyr^{607}$, phosphorylated Tyr^{607} in PI3K p85α

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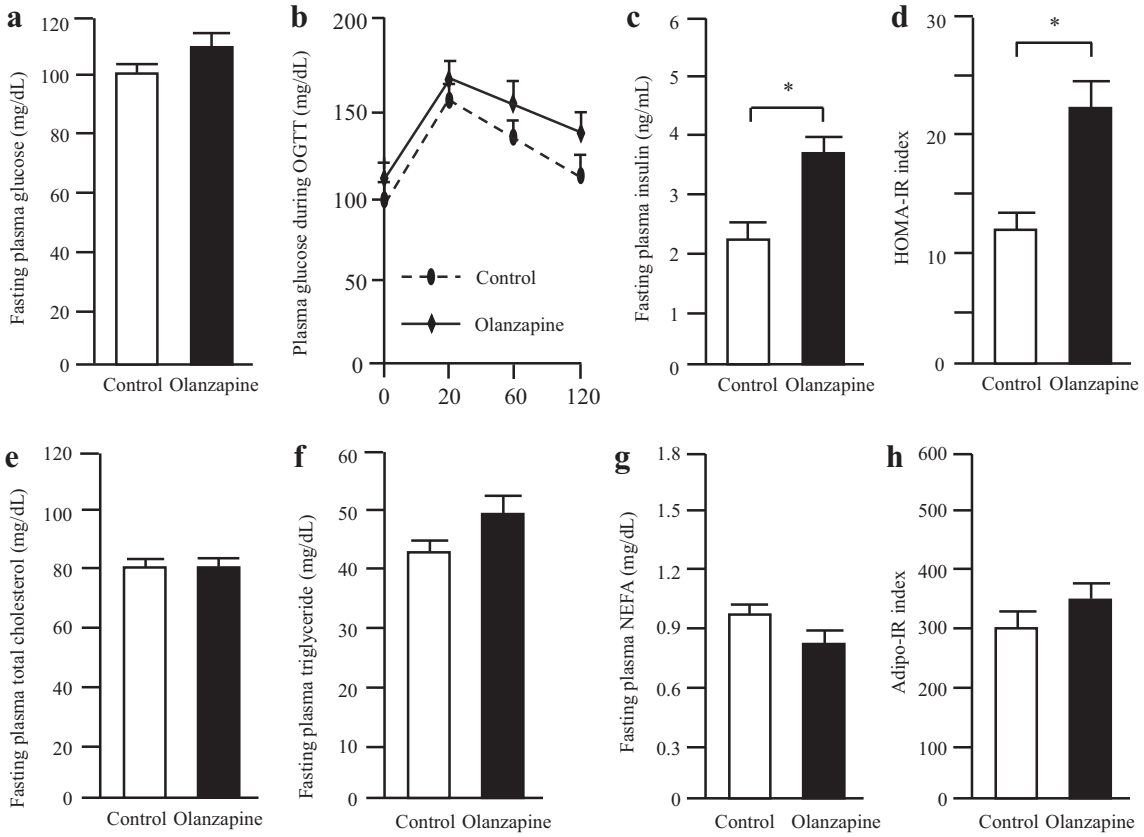

Fig. 1. Plasma glucose concentrations at the baseline (a) and during OGTT (b), fasting insulin concentration (c), the HOMA-IR index (d), total cholesterol (e), triglyceride (f), NEFA (g) and the adipose tissue insulin resistance (Adipo-IR) index (h) in olanzapine-treated and the corresponding control groups. Data are means ± SEM (n = 8 each group). *$P < 0.05$.

demonstrated that acute and chronic treatment with olanzapine induces systemic and hepatic insulin resistance [2,3,5–14], hypertriglyceridemia and fatty liver [2–4] in rodents and humans. However, the underlying mechanisms are still largely unknown.

The liver, one of the major target organs of insulin, plays a central role in lipid and glucose metabolism [15]. Hepatic insulin resistance is a common feature of obesity and type 2 diabetes [16,17]. Insulin receptor substrate (IRS)-1 and IRS-2 are necessary for many insulin responses to various stimuli [18]. Impairment of the IRS signals is associated with induction and aggravation of insulin resistance [19,20]. Phosphoinositide 3-kinase (PI3K)s, a family of intracellular signal transducer enzymes, are associated with an extraordinarily diverse group of cellular functions [21]. The serine/threonine kinase Akt (protein kinase B) is a downstream target enzyme of PI3K signaling. Akt is a mostly important and versatile protein kinase. It plays diverse cellular roles in cell signaling downstream of growth factors, cytokines, and other cellular stimuli-mediated cell survival, growth, proliferation, angiogenesis, metabolism, and migration [22]. The IRS/PI3K/Akt signaling pathway has been suggested to play an important role in regulating insulin signaling and lipid metabolism [21–23].

In the context, the present study investigated the role of the IRS/PI3K/Akt signaling pathway in olanzapine-induced hepatic insulin resistance in male rats.

2. Materials and methods

2.1. Rats, diet and experimental protocol

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

It has been reported that treatment with antipsychotics including olanzapine results in an increase in body weight in female, but not male rats [2,24,25]. To exclude the interference of body weight change with glucose and lipid metabolism, male rats were used in this study. Male Sprague–Dawley rats weighing 150-180 g and standard laboratory chow were purchased from the laboratory animal center, Southern Medical University, China. The rats were housed in a SPF-degree facility (22 ± 1 °C, 55 ± 5% relative humidity) with a 12-h light/dark cycle. Rats were free access to tap water and the chow for 1 week before the start of experiment.

It has been reported that acute and chronic treatments with olanzapine from 2.5 to 15 mg/kg/day cause insulin resistance and excessive hepatic lipid accumulation [2–14,26]. Based on the previous reports and our preliminary experiments, 5 mg/kg was chosen as the dose of olanzapine in the present study. Sixteen rats were grouped as vehicle control group and olanzapine-treated group (n = 8 each). Olanzapine group was given olanzapine (5 mg/kg, Civi Chem & Applications Pvt. Ltd., Shanghai, China, suspended in 5% Gum Arabic solution, by a gavage method, once daily for consecutive 8 weeks), while the control group was given 5% Gum Arabic alone. To decrease the interference of stress, two animals only were housed in a cage. All rats were free access to the diet and tap water. The consumed chow per cage was weighed daily. At week 7, overnight-fasting blood sample was collected for determination of plasma concentrations of glucose (kit from Shanghai Rongsheng Biotech, Shanghai, China), insulin (kit from Shanghai Mlbio Biotechnology, Shanghai, China), total cholesterol and triglyceride (kits from Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and non-esterified fatty acid (NEFA) (NEFA-C kit, Wako, Osaka, Japan) using enzymatic methods or by ELISA. The indexes of the homeostasis model assessment of insulin resistance (HOMA-IR) and adipose tissue

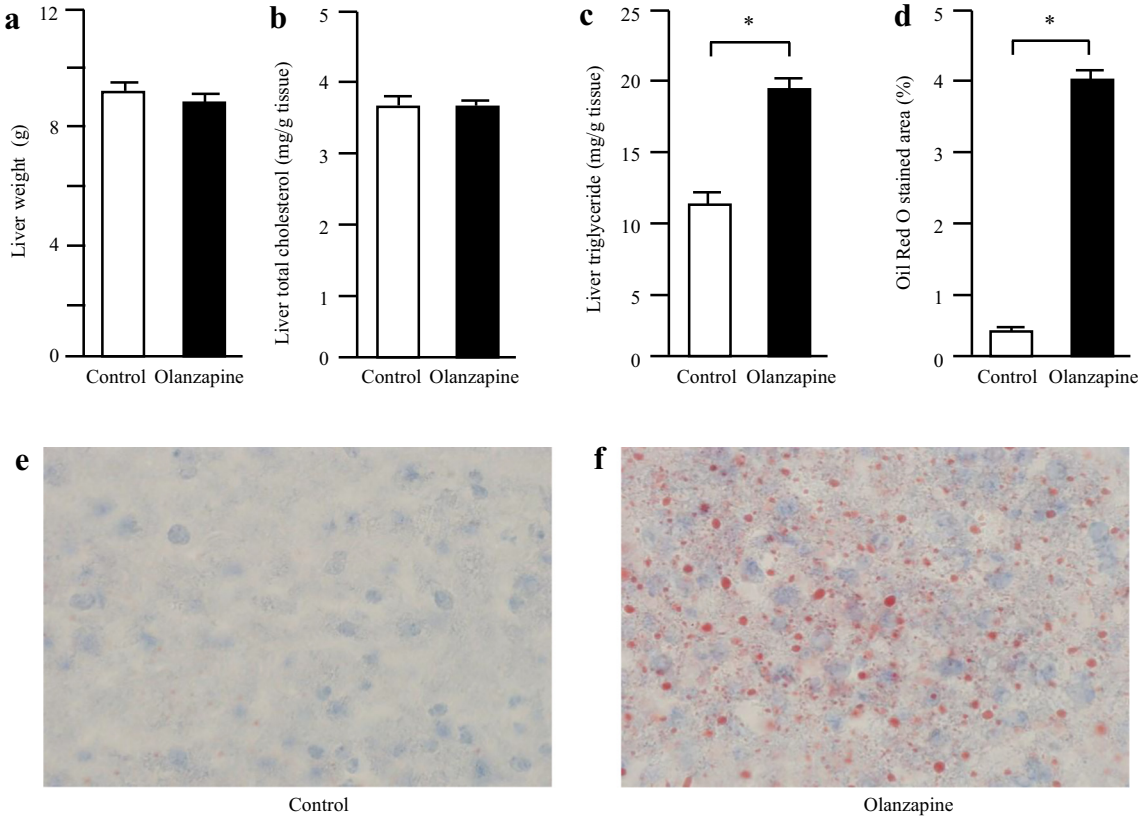

Fig. 2. Liver weight (a), liver total cholesterol content (b), liver triglyceride content (c), Oil Red O-stained area (d), and representative images showing hepatic fatty droplet accumulation (Oil Red O staining, E and F, X 1000) in olanzapine-treated and their corresponding control groups. Data are means ± SEM (n = 8 each group). *$P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

insulin resistance (Adipo-IR) were calculated according to the following formulas: [fasted insulin (μIU/mL) × fasted glucose (mM)]/22.5 and [fasted insulin (mmol/L) × fasted NEFA (pmol/L)], respectively [27]. Immediately followed blood sampling, an oral glucose tolerance test was performed. Briefly, a glucose solution (2 g/kg in 5 mL) was given by oral gavage. Blood samples were collected again 20, 60 and 120 min after glucose feeding for determination of plasma glucose concentrations. At week 8, overnight-fasting rats (still free access to water), were weighed and euthanized by prompt dislocation of the neck vertebra under anesthesia. Liver was dissected and weighed. Segments of liver were flash frozen in liquid nitrogen and stored at −80 °C for subsequent determination of triglyceride and total cholesterol contents, and gene/protein expression.

2.2. Determination of hepatic total cholesterol and triglyceride contents

The determination was carried out as described previously [25]. Briefly, a liver section was homogenized and extracted with isopropanol (100 mg/2 mL). Total cholesterol and triglyceride concentrations in the supernatant (3000 rpm centrifugation) were determined enzymatically (kit from Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The hepatic total cholesterol and triglyceride contents were calculated accordingly.

2.3. Histological examination

Six-micron frozen section of the liver was stained with the Oil Red O method, and the stained area (fatty droplet accumulation) was determined using an ImageJ 1.43 analyzing system. The ratio (%) of the Oil Red O-stained area (Red) to total tissue area was calculated accordingly.

2.4. Real-time PCR

Guided by the manufacturer's instructions, hepatic cDNA was synthesized (M-MLV RTase cDNA Synthesis, Kits from Takara, Dalian, China). Real time PCR was performed with the LIGHTCYCLER 480 Real-Time PCR Detection System (LIGHTCYCLER 480, Roche, Germany) using the SYBR® Premix Ex Taq™ II (Takara, Dalian, China). The sequences of primers are shown in Supplementary materials: Table 1. mRNA expression was normalized against the corresponding control gene β-actin. The expression of the target gene in the corresponding control was arbitrarily assigned a value of 1.

2.5. Western blot

Individually-isolated total protein (30 μg) was separated on 8% SDS-polyacrylamide gels and transferred to Polyvinylidene Fluoride Membrane. Total IRS (tIRS)-1, phosphorylated IRS-1 at Ser^{307} ($pSer^{307}$), total IRS (tIRS)-2, phosphorylated IRS-2 at Ser^{731} ($pSer^{731}$), sterol regulatory element-binding protein (SREBP)-1c and carbohydrate-response element-binding protein (ChREBP), total phosphoinositide 3-kinase (tPI3K) p85α, phosphorylated PI3K p85α at Tyr^{607} ($pTyr^{607}$), total Akt (tAkt), phosphorylated Akt at Ser^{473} ($pSer^{473}$), total mammalian target of rapamycin (mTOR) (tmTOR), and phosphorylated Ser^{2448} in mTOR ($pSer^{2448}$) (dilutions and the makers of the first antibodies are shown in Supplementary materials: Table 2) were detected with rabbit polyclonal antibody. Detection of signals was performed using the ECL Western blot detection kit (Proteintech Group, Inc., Chicago, USA) with anti-rabbit horseradish peroxidase-conjugated IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as the second antibody. Polyclonal rabbit β-actin antibody (Cell Signaling Technologies, Beverly, MA, USA) was used as the loading control to normalize the

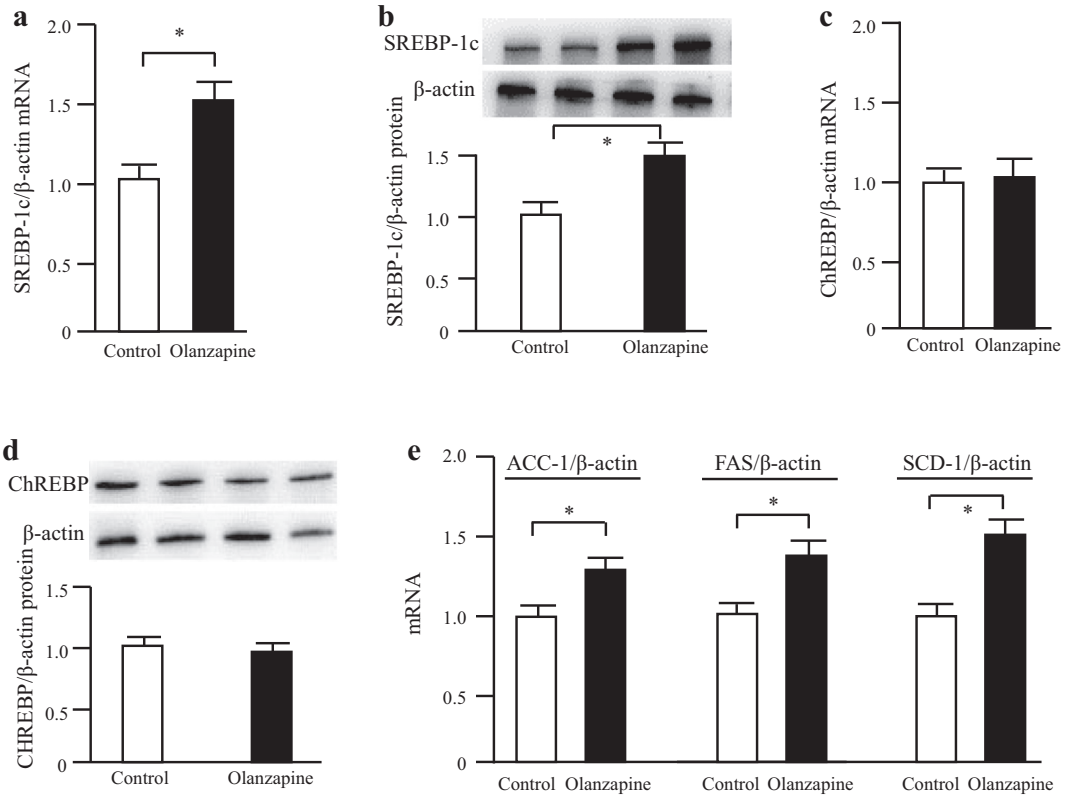

Fig. 3. Hepatic expression of mRNAs by real-time PCR and proteins by Western blot of sterol regulatory element-binding protein (SREBP)-1c (a and b) and carbohydrate response element binding protein (ChREBP) (c and d), and mRNAs encoding acetyl-CoA carboxylase (ACC)-1, fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD)-1 (E) in olanzapine-treated and their corresponding control groups. The corresponding control levels were arbitrarily assigned a value of 1. Data are means ± SEM (n = 8 each group). *$P < 0.05$.

signal obtained for proteins. The density of immunoreactive bands was measured using ImageJ 1.43. The corresponding control levels were arbitrarily assigned a value of 1.

2.6. Data analysis

All digital data are expressed as mean ± SEM. All statistical analyses were performed using StatView (Version 5.0.1) for Windows (SAS Institute Inc., Cary, NC, USA). Student's *t*-test was used to evaluate whether differences between the treatment group and the corresponding control group were significant. $P < 0.05$ indicates statistical significance.

3. Results

3.1. Chow intake, body weight, and blood biochemical parameters in rats

Administration of olanzapine for 8 weeks did not affect chow intake and body weight (Supplementary materials: Table 3).

Olanzapine did not affect plasma glucose concentrations either under fasting condition (Fig. 1a) or during OGTT (Fig. 1b) in rats. However, it significantly increased plasma insulin concentration (Fig. 1c) and the HOMA-IR index (Fig. 1d). Treatment with olanzapine did not significantly affect plasma concentrations of total cholesterol (Fig. 1e), triglyceride (Fig. 1f), NEFA (Fig. 1g) and the Adipo-IR index (Fig. 1h).

3.2. Hepatic parameters in rats

Treatment with olanzapine did not significantly affect liver weight (Fig. 2a) and total cholesterol content (Fig. 2b), but it substantially increased triglyceride content (Fig. 2c). Histological examination showed a substantial increase in Oil Red O-stained area in the hepatocytes of olanzapine-treated rats, compared to that in the corresponding controls (Fig. 2d–f).

3.3. Hepatic gene/protein expression profile in rats

Treatment with olanzapine substantially upregulated SREBP-1c mRNA (Fig. 3a) and protein (Fig. 3b and Supplementary materials: Fig. 1) expression. However, it did not induce change in hepatic mRNA (Fig. 3c) and protein (Fig. 3d and Supplementary materials: Fig. 1) expression of ChREBP. Hepatic mRNA expression of acetyl-CoA carboxylase (ACC)-1, fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD)-1 (Fig. 3e) was upregulated by olanzapine.

Western blot analysis also showed that treatment with olanzapine did not affect protein expression of tIRS-1 (Fig. 4a and Supplementary materials: Fig. 1) and tIRS-2 (Fig. 4d and Supplementary materials: Fig. 1), but it significantly increased $pSer^{307}$ in IRS-1 (Fig. 4b and Supplementary materials: Fig. 1) and $pSer^{731}$ in IRS-2 (Fig. 4e and Supplementary materials: Fig. 1) at baseline (fasting condition). The increases in phosphorylated proteins contributed to upregulation of the ratios of $pSer^{307}$ in IRS-1 to tIRS-1 (Fig. 4c) and $pSer^{731}$ in IRS-2 to tIRS-2 (Fig. 4f).

Although tPI3K p85α (Fig. 5a and Supplementary materials: Fig. 2) and tAkt (Fig. 5d and Supplementary materials: Fig. 2) protein levels remained unchanged, $pTyr^{607}$ in PI3K p85α (Fig. 5b and Supplementary materials: Fig. 2) and $pSer^{473}$ in Akt (Fig. 5e and Supplementary materials: Fig. 2) were upregulated, the ratios of $pTyr^{607}$ in PI3K p85α to tPI3K p85α (Fig. 5c) and $pSer^{473}$ in Akt to tAkt (Fig. 5f) were also increased by olanzapine treatment. Similarly, olanzapine elevated $pSer^{2448}$ in mTOR protein level (Fig. 6b and Supplementary materials:

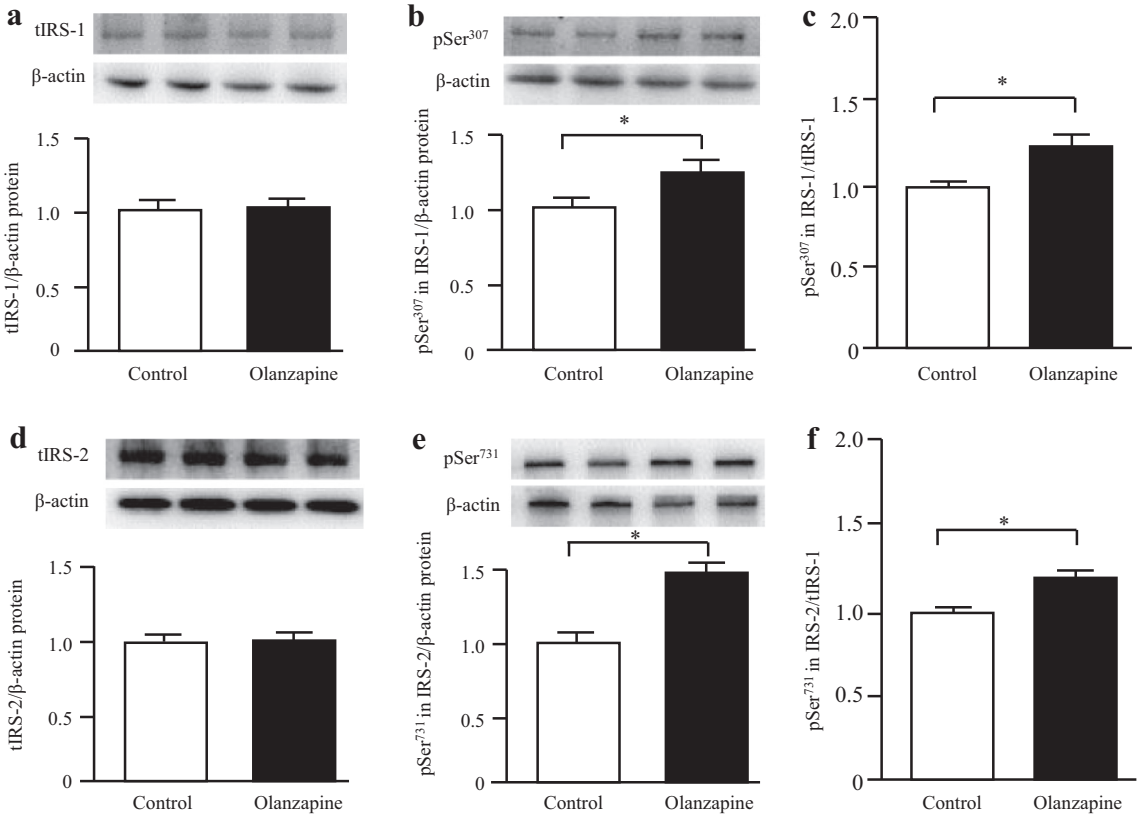

Fig. 4. Hepatic expression of total insulin receptor substrate (IRS)-1 (tIRS-1, a), phosphorylated Ser^{307} in IRS-1 ($pSer^{307}$ in IRS-1, b), the ratio of $pSer^{307}$ in IRS-1 to tIRS-1 (c), total IRS-2 (tIRS-2, d), Ser^{731} in IRS-2 ($pSer^{731}$ in IRS-2, e) and the ratio of $pSer^{731}$ in IRS-1 to tIRS-1 (f) in olanzapine-treated and their corresponding control groups. The corresponding control levels were arbitrarily assigned a value of 1. Data are means ± SEM (n = 8 each group). *$P < 0.05$.

Fig. 2), but it did not affect tmTOR protein expression (Fig. 6a and Supplementary materials: Fig. 2). The ratio of $pSer^{2448}$ in mTOR to tmTOR was increased (Fig. 6c).

4. Discussion

At the initial stage of insulin resistance, an increase in insulin secretion of β-cells with compensatory mechanisms results in hyperinsulinemia, which keeps blood glucose concentration still at the normal level. The HOMA-IR index has been suggested to evaluate hepatic insulin sensitivity [27]. Hyperinsulinemia may trigger hepatic de novo lipid synthesis by activating SREBP-1c, one of the key transcript factors; thus excessive lipid accumulation in the hepatocytes is strongly associated with the development of hepatic insulin resistance [28,29]. In the present study, chronic treatment with olanzapine strikingly increased fasting plasma insulin concentration and the HOMA-IR index, although it did not significantly affect plasma glucose concentrations at the baseline and during OGTT in male rats. Furthermore, olanzapine also induced excessive hepatic triglyceride and fatty droplet accumulation, accompanied by activation of hepatic SREBP-1c and its downstream lipogenic genes ACC-1, FAS and SCD-1. Thus, the results suggest that chronic treatment with olanzapine induces hepatic insulin resistance in male rats.

IRS signal impairment is closely associated with development of insulin resistance. Generally, serine phosphorylation is deleterious to IRS signaling [30]. Serine phosphorylation of IRS-1 is increased in insulin-resistant states, and plays a key role in the development of insulin resistance [31]. Ser^{307} in IRS-1 has been indicated as a molecular indicator of insulin resistance when the serine residues of IRS-1 are phosphorylated in response to stimuli [32]. Increased $pSer^{307}$ in IRS-1 diminishes insulin signaling [32]. In addition to IRS-1, IRS-2 is also a major player of insulin action in the liver [33]. Phosphorylation at Ser^{731} is counter regulatory, thus used as a marker to evaluate IRS-2 disruption-mediated impaired insulin signaling [34]. In the present study, treatment with olanzapine substantially increased hepatic $pSer^{307}$ in IRS-1, $pSer^{731}$ in IRS-2, and the ratios of $pSer^{307}$ to tIRS-1 and $pSer^{731}$ to tIRS-2. Thus, these results suggest the role of olanzapine-induced IRS signal impairment in development of hepatic insulin resistance.

Accumulated evidence has suggested that the PI3K/AKT signaling pathway is required for normal glucose and lipid metabolism, and the imbalance of the pathway leads to development of obesity and type 2 diabetes as the results of insulin resistance [35]. PI3K p85α regulatory subunit is expressed ubiquitously and recognized as an important negative regulator of insulin action via phosphorylating IRS proteins [31]. It suppresses insulin action in the liver, and plays an important role in maintaining hepatic lipid homeostasis by regulating SREBP-1c activities [23,34,36]. Overexpression of this subunit results in insulin resistance [31,37]. It has been demonstrated both in vitro and in vivo that Tyr^{607} is one of the tyrosine phosphorylation sites of PI3K p85α subunit by insulin receptor [38]. Akt is a downstream kinase of PI3K [22]. The regulatory effects of PI3K on hepatic glucose and lipid metabolism are mediated by Akt [36]. Akt is also associated with mTOR to form the Akt-mTOR axis that has been suggested as central to the insulin signaling cascade [39]. Basal Akt phosphorylation at Ser^{473} has been reported to be elevated in the livers of obese insulin-resistant mice [40] and in human primary myotubes overexpressing adipose triglyceride lipase contributes to insulin resistance [41]. It has been demonstrated that olanzapine increases the PI3K pathway-mediated Akt phosphorylation at Ser^{473} in pheochromocytoma cells [42]. Ser^{2448} phosphorylation has been suggested to assess mTOR kinase activation or as a proxy measure of induction of the mTOR pathway in a large number of

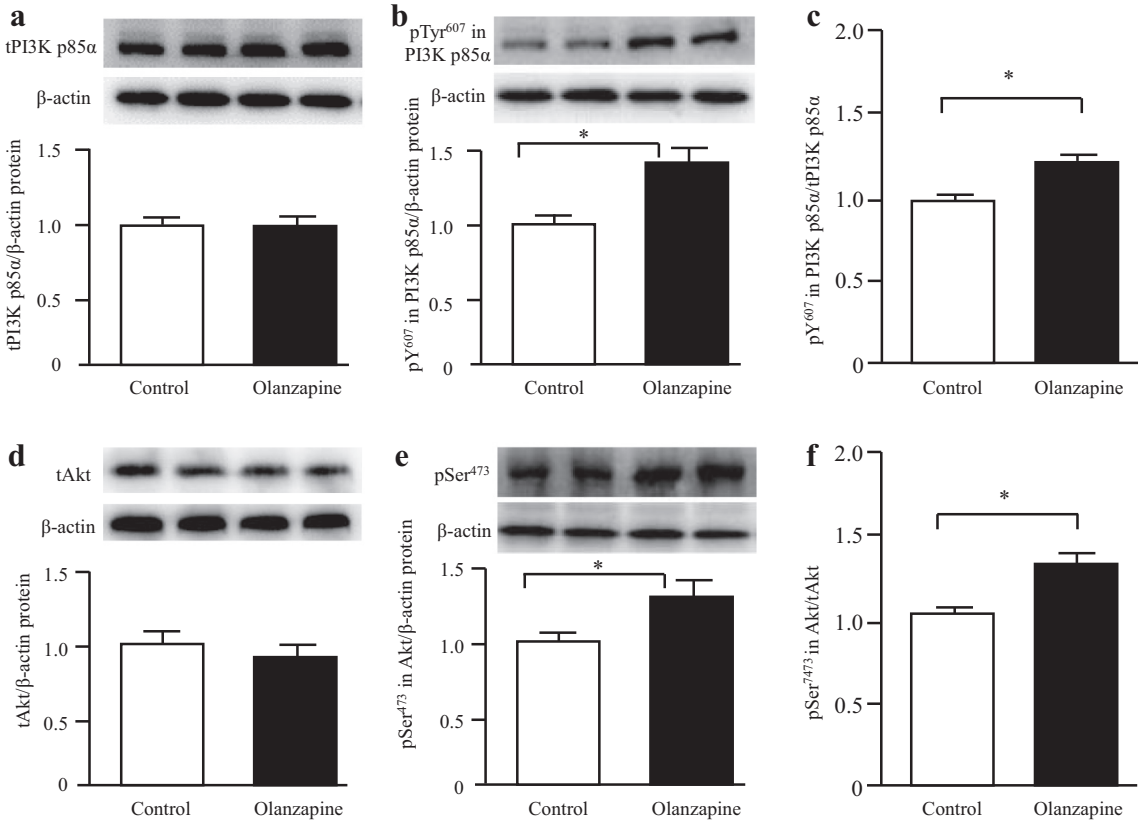

Fig. 5. Hepatic expression of total phosphoinositide 3-kinase (PI3K) p85α (tPI3K p85α, a), phosphorylated Tyr^{607} in PI3K p85α (pTyr^{607}, b), the ratio of pTyr^{607} in PI3K p85α to tPI3K p85α (c), total Akt (tAkt, d), phosphorylated Ser^{473} in Akt (pSer^{473}, e), and the ratio of pSer^{473} in Akt to total Akt (tAkt, f) in olanzapine-treated and their corresponding control groups. The corresponding control levels were arbitrarily assigned a value of 1. Data are means ± SEM (n = 8 each group). *$P < 0.05$.

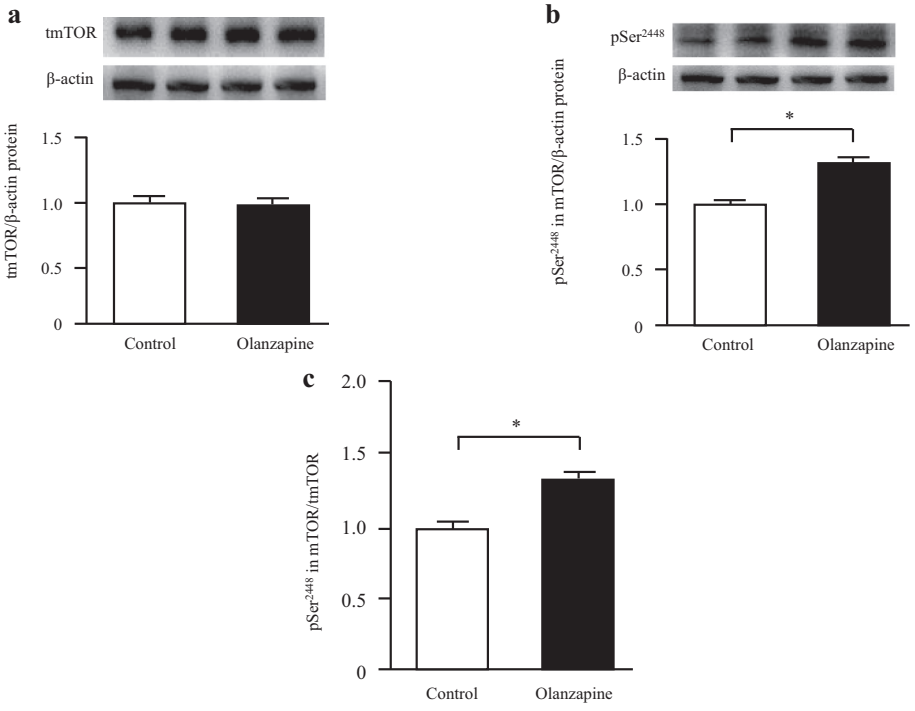

Fig. 6. Hepatic expression of total mammalian target of rapamycin (mTOR) protein (tmTOR, a), phosphorylated Ser^{2448} (pSer^{2448}) in mTOR (b), and the ratio of pSer^{2448} in mTOR to tmTOR (c) in olanzapine-treated and their corresponding control groups. The corresponding control levels were arbitrarily assigned a value of 1. Data are means ± SEM (n = 8 each group). *$P < 0.05$.

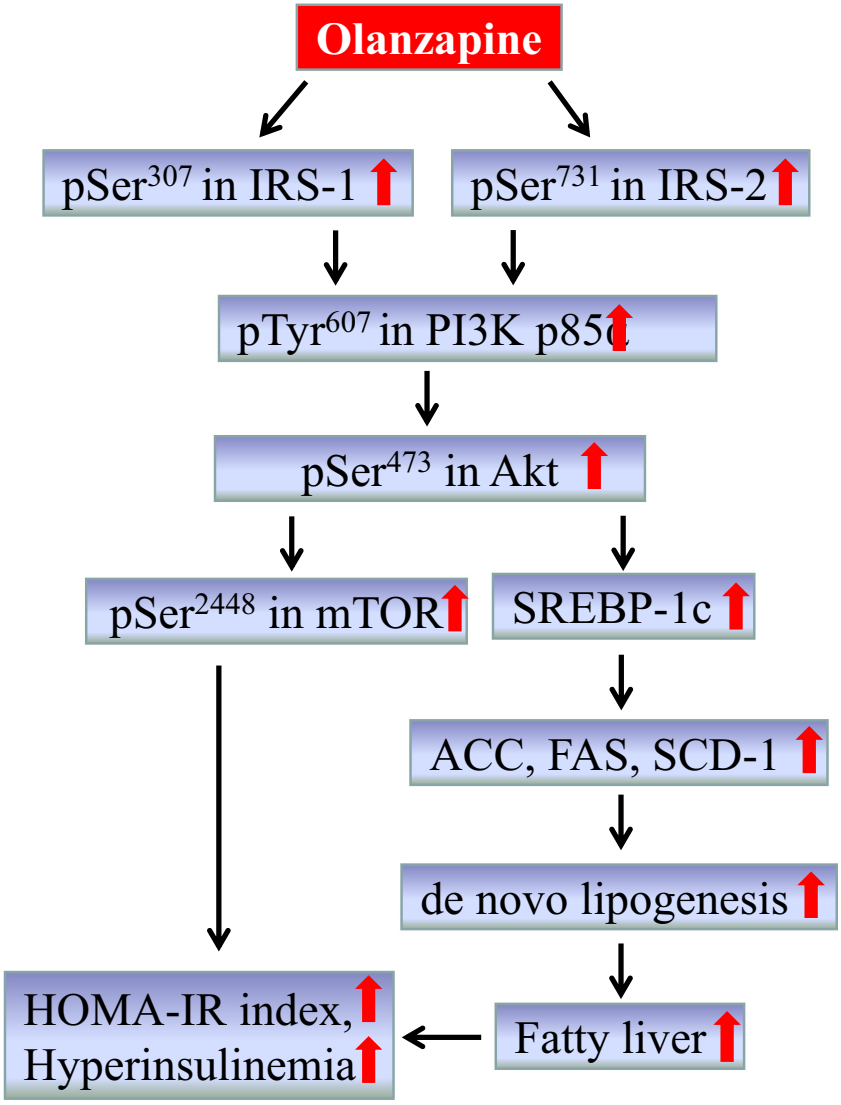

Fig. 7. Overview of the mechanisms underlying olanzapine-induced hepatic insulin resistance in male rats.

studies [43]. In the present study, treatment with olanzapine substantially upregulated hepatic pTyr^{607} in PI3K p85α, pSer^{473} in Akt, and their ratios to the total proteins. These results demonstrated that olanzapine enhanced the PI3K p85α subunit and Akt phosphorylation. Furthermore, olanzapine upregulated downstream effector pSer^{2448} in mTOR and its ratio to mTOR total protein. It appears that the PI3K/Akt signaling is involved in IRS disruption-mediated hepatic insulin resistance in olanzapine-loaded rats.

5. Conclusion

Taken together, the present results demonstrate that the IRS/PI3K/Akt signaling pathway mediates olanzapine-induced hepatic abnormalities of lipid and glucose metabolism in male rats (see the overview of the underlying mechanisms in Fig. 7). Our findings may provide better understanding of the antipsychotic-induced metabolic adverse effects.

Disclosure statement

All the authors declared no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2018.12.015.

References

[1] M.A. Cornier, D. Dabelea, T.L. Hernandez, R.C. Lindstrom, A.J. Steig, N.R. Stob, R.E. Van Pelt, H. Wang, R.H. Eckel, The metabolic syndrome, Endocr. Rev. 29 (2008) 777–822, https://doi.org/10.1210/er.2008-0024.
[2] J.S. Ballon, U. Pajvani, Z. Freyberg, R.L. Leibel, J.A. Lieberman, Molecular pathophysiology of metabolic effects of antipsychotic medications, Trends Endocrinol. Metab. 25 (2014) 593–600, https://doi.org/10.1016/j.tem.2014.07.004.
[3] L.E. Rojo, P.A. Gaspar, H. Silva, L. Risco, P. Arena, K. Cubillos-Robles, B. Jara, Metabolic syndrome and obesity among users of second generation antipsychotics: a global challenge for modern psychopharmacology, Pharmacol. Res. 10 (2015) 74–85, https://doi.org/10.1016/j.phrs.2015.07.022.
[4] X. Liu, J. Lian, C.H. Hu, C. Deng, Betahistine co-treatment ameliorates dyslipidemia

induced by chronic olanzapine treatment in rats through modulation of hepatic AMPKα-SREBP-1 and PPARα-dependent pathways, Pharmacol. Res. 100 (2015) 36–46, https://doi.org/10.1016/j.phrs.2015.07.023.
[5] M. Ader, S.P. Kim, K.J. Catalano, V. Ionut, K. Hucking, J.M. Richey, M. Kabir, R.N. Bergman, Metabolic dysregulation with atypical antipsychotics occurs in the absence of underlying disease: a placebo-controlled study of olanzapine and risperidone in dogs, Diabetes 54 (2005) 862–871, https://doi.org/10.2337/diabetes.54.3.862.
[6] A.F. Chintoh, S.W. Mann, T.K. Lam, A. Giacca, G. Remington, Insulin resistance following continuous, chronic olanzapine treatment: an animal model, Schizophr. Res. 104 (2008) 23–30, https://doi.org/10.1016/j.schres.2008.06.006.
[7] A.F. Chintoh, S.W. Mann, L. Lam, C. Lam, T.A. Cohn, P.J. Fletcher, J.N. Nobrega, A. Giacca, G. Remington, Insulin resistance and decreased glucose-stimulated insulin secretion after acute olanzapine administration, J. Clin. Psychopharmacol. 28 (2008) 494–499, https://doi.org/10.1097/JCP.0b013e318184b4c5.
[8] R. Coccurello, D. Brina, A. Caprioli, R. Conti, O. Ghirardi, F. Schepis, A. Moles, 30 days of continuous olanzapine infusion determines energy imbalance, glucose intolerance, insulin resistance, and dyslipidemia in mice, J. Clin. Psychopharmacol. 29 (2009) 576–583, https://doi.org/10.1097/JCP.0b013e3181bfe13e.
[9] P.J. Martins, M. Haas, S. Obici, Central nervous system delivery of the antipsychotic olanzapine induces hepatic insulin resistance, Diabetes 59 (2010) 2418–2425, https://doi.org/10.2337/db10-0449.
[10] H.N. Boyda, R.M. Procyshyn, L. Tse, E. Hawkes, C.H. Jin, C.C. Pang, W.G. Honer, A.M. Barr, Differential effects of 3 classes of antidiabetic drugs on olanzapine-induced glucose dysregulation and insulin resistance in female rats, J. Psychiatry Neurosci. 37 (2012) 407–415, https://doi.org/10.1503/jpn.110140.
[11] K.L. Teff, M.R. Rickels, J. Grudziak, C. Fuller, H.L. Nguyen, K. Rickels, Antipsychotic-induced insulin resistance and postprandial hormonal dysregulation independent of weight gain or psychiatric disease, Diabetes 62 (2013) 3232–3240, https://doi.org/10.2337/db13-0430.
[12] K.L. Teff, K. Rickels, E. Alshehabi, M.R. Rickels, Metabolic impairments precede changes in hunger and food intake following short-term administration of second-generation antipsychotics, J. Clin. Psychopharmacol. 35 (2015) 579–582, https://doi.org/10.1097/JCP.0000000000000393.
[13] G.J. Remington, C. Teo, V. Wilson, A. Chintoh, M. Guenette, Z. Ahsan, A. Giacca, M.K. Hahn, Metformin attenuates olanzapine-induced hepatic, but not peripheral insulin resistance, J. Endocrinol. 227 (2015) 71–81, https://doi.org/10.1530/JOE-15-0074.
[14] M.R. Rickels, E.M. Perez, A.J. Peleckis, E. Alshehabi, H.L. Nguyen, D. Stefanovski, K. Rickels, K.L. Teff, Contribution of parasympathetic muscarinic augmentation of insulin secretion to olanzapine-induced hyperinsulinemia, Am. J. Physiol. Endocrinol. Metab. 315 (2018) E250–E257, https://doi.org/10.1152/ajpendo.00315.2017.
[15] R.A. Haeusler, D. Accili, The double life of IRS, Cell Metab. 8 (2008) 7–9, https://doi.org/10.1016/j.cmet.2008.06.010.
[16] B.B. Kahn, J.S. Flier, Obesity and insulin resistance, J. Clin. Invest. 106 (2000) 473–481, https://doi.org/10.1172/JCI10842.
[17] D.B. Savage, K.F. Petersen, G.I. Shulman, Disordered lipid metabolism and the pathogenesis of insulin resistance, Physiol. Rev. 87 (2007) 507–520, https://doi.org/10.1152/physrev.00024.2006.
[18] M.F. White, Insulin signaling in health and disease, Science 302 (2003) 1710–1711, https://doi.org/10.1126/science.1092952.
[19] H.K. Karlsson, J.R. Zierath, Insulin signaling and glucose transport in insulin resistant human skeletal muscle, Cell. Biochem. Biophys. 48 (2007) 103–113, https://doi.org/10.1007/s12013-007-0030-9 (doi:10.2337/dc09-S302).
[20] R.A. De Fronzo, D. Tripathy, Skeletal muscle insulin resistance is the primary defect in type 2 diabetes, Diabetes Care 32 (Suppl. 2) (2009) 157–163.
[21] J.R. Krycer, L.J. Sharpe, W. Luu, A.J. Brown, The Akt-SREBP nexus: cell signaling meets lipid metabolism, Trends Endocrinol. Metab. 21 (2010) 268–276, https://doi.org/10.1016/j.tem.2010.01.001.
[22] B.D. Manning, L.C. Cantley, AKT/PKB signaling: navigating downstream, Cell 129 (2007) 1261–1274, https://doi.org/10.1016/j.cell.2007.06.009.
[23] T.I. Jeon, T.F. Osborne, SREBPs: metabolic integrators in physiology and metabolism, Trends Endocrinol. Metab. 23 (2012) 65–72, https://doi.org/10.1016/j.tem.2011.10.004.
[24] A. Stefanidis, M.J. Watt, M.A. Cowley, B.J. Oldfield, Prevention of the adverse effects of olanzapine on lipid metabolism with the antiepileptic zonisamide, Neuropharmacology 123 (2017) 55–66, https://doi.org/10.1016/j.neuropharm.2017.04.010.
[25] X. Zhou, L. Ren, Z. Yu, X. Huang, Y. Li, C. Wang, The antipsychotics sulpiride induces fatty liver in rats via phosphorylation of insulin receptor substrate-1 at serine 307-mediated adipose tissue insulin resistance, Toxicol. Appl. Pharmacol. 345 (2018) 66–74, https://doi.org/10.1016/j.taap.2018.02.023.
[26] X. Liu, Z. Wu, J. Lian, C.H. Hu, X.F. Huang, C. Deng, Time-dependent changes and potential mechanisms of glucose-lipid metabolic disorders associated with chronic clozapine or olanzapine treatment in rats, Sci. Rep. 7 (2017) 2762, , https://doi.org/10.1038/s41598-017-02884-w.
[27] B.A. Neuschwander-Tetri, Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites, Hepatology 52 (2010) 774–788, https://doi.org/10.1002/hep.23719.
[28] C. Postic, J. Girard, Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice, J. Clin. Invest. 118 (2008) 829–838, https://doi.org/10.1172/JCI34275.
[29] P. Ferré, F. Foufelle, Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c, Diabetes Obes. Metab. 12 (2010) 83–92, https://doi.org/10.1111/j.1463-1326.2010.01275.x.
[30] K. Paz, R. Hemi, D. Leroith, A. Karasik, E. Elhanany, H. Kanety, Y. Zick, A molecular basis for insulin resistance: elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation, J. Biol. Chem. 272 (1997) 29911–29918.
[31] C.M. Taniguchi, B. Emanuelli, C.R. Kahn, Critical nodes in signaling pathways: insights into insulin action, Nat. Rev. Mol. Cell Biol. 7 (2006) 85–96, https://doi.org/10.1038/nrm1837.
[32] V. Aguirre, E.D. Werner, J. Giraud, Y.H. Lee, S.E. Shoelson, M.F. White, Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action, J. Biol. Chem. 277 (2002) 1531–1537, https://doi.org/10.1074/jbc.M101521200.
[33] S.B. Biddinger, C.R. Kahn, From mice to men: insights into the insulin resistance syndromes, Annu. Rev. Physiol. 68 (2006) 123–158, https://doi.org/10.1109/TCE.2007.4429216.
[34] R.S. Rector, E.M. Morris, S. Ridenhour, G.M. Meers, F.F. Hsu, J. Turk, J.A. lbdah, Selective hepatic insulin resistance in a murine model heterozygous for a mitochondrial trifunctional protein defect, Hepatology 57 (2013) 2213–2223, https://doi.org/10.1002/hep.26285.
[35] X. Huang, G. Liu, J. Guo, Z. Su, The PI3K/AKT pathway in obesity and type 2 diabetes, Int. J. Biol. Sci. 14 (2018) 1483–1496, https://doi.org/10.7150/ijbs.27173.
[36] C.M. Taniguchi, T. Kondo, M. Sajan, J. Luo, R. Bronson, T. Asano, R. Farese, L.C. Cantley, C.R. Kahn, Divergent regulation of hepatic glucose and lipid metabolism by phosphoinositide 3-kinase via Akt and PKClambda/zeta, Cell Metab. 3 (2006) 343–353, https://doi.org/10.1016/j.cmet.2006.04.005.
[37] C.M. Taniguchi, T.T. Tran, T. Kondo, J. Luo, K. Ueki, L.C. Cantley, C.R. Kahn, Phosphoinositide3-kinase regulatory subunit p85alpha suppresses insulin action via positive regulation of PTEN, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12093–12097, https://doi.org/10.1073/pnas.0604628103.
[38] H. Hayashi, Y. Nishioka, S. Kamohara, F. Kanai, K. Ishii, Y. Fukui, F. Shibasaki, T. Takenawa, H. Kido, N. Katsunuma, The alpha-type 85-kDa subunit of phosphatidylinositol 3-kinase is phosphorylated at tyrosines 368, 580, and 607 by the insulin receptor, J. Biol. Chem. 268 (1993) 7107–7117.
[39] P.M. Titchenell, W.J. Quinn, M. Lu, Q. Chu, W. Lu, C. Li, H. Chen, B.R. Monks, J. Rabinowitz, J.D. Chen, M.J. Birnbaum, Direct hepatocyte insulin signaling is required for lipogenesis but is dispensable for the suppression of glucose production, Cell Metab. 23 (2016) 1154–1166, https://doi.org/10.1016/j.cmet.2016.04.022.
[40] H.Y. Liu, T. Hong, G.B. Wen, J.M. Han, D.G. Zuo, Z.Q. Liu, W.H. Cao, Increased basal level of Akt-dependent insulin signaling may be responsible for the development of insulin resistance, Am. J. Physiol. Endocrinol. Metab. 297 (2009) E898–E906, https://doi.org/10.1152/ajpendo.00374.2009.
[41] P.M. Badin, K. Louche, A. Mairal, G. Liebisch, G. Schmitz, A.C. Rustan, S.R. Smith, D. Langin, C. Moro, Altered skeletal muscle lipase expression and activity contribute to insulin resistance in humans, Diabetes 60 (2011) 1734–1742, https://doi.org/10.2337/db10-1364.
[42] X.H. Lu, R.J. Bradley, D.S. Dwyer, Olanzapine produces trophic effects in vitro and stimulates phosphorylation of Akt/PKB, ERK1/2, and the mitogen-activated protein kinase p38, Brain Res. 1011 (2004) 58–68, https://doi.org/10.1016/j.brainres.2004.03.018.
[43] V.C. Figueiredo, J.F. Markworth, D. Cameron-Smith, Considerations on mTOR regulation at serine 2448: implications for muscle metabolism studies, Cell. Mol. Life Sci. 74 (2017) 2537–2545, https://doi.org/10.1007/s00018-017-2481-5.