Role of thiazolidinediones, insulin sensitizers, in non-alcoholic fatty liver disease

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ABSTRACT

The prevalence of metabolic syndrome, obesity and insulin resistance has become an epidemic in the world. A strong association exists between metabolic syndrome and non-alcoholic fatty liver disease (NAFLD), though the etiology of NAFLD is still unclear. This close association leads to numerous clinical studies to investigate the effects of insulin sensitizers, thiazolidinediones (TZDs), on hepatic fat accumulation. Thiazolidinediones affect glucose and lipid metabolism in insulin-sensitive tissues, which in turn reduces the lipid content in the liver by modulating several mediators. In the present review, we discuss key modulators – adiponectin and sirtulin-adenosine monophosphate activated protein kinase signaling – as the mechanisms responsible for NAFLD related to metabolic syndrome. (J Diabetes Invest, doi: 10.1111/jdi.12107, 2013)

KEY WORDS: Insulin resistance, Non-alcoholic fatty liver disease, Thiazolidinediones

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease representing fat accumulation in the liver of a non-alcoholic subject. It includes a broad spectrum of hepatic disorders ranging from simple triglyceride (TG) accumulation in hepatocytes (hepatic steatosis) to non-alcoholic steatohepatitis (NASH), which is characterized by the additional presence of inflammation and injury. Non-alcoholic steatohepatitis can progress to cirrhosis, decompensated liver disease and hepatocellular carcinoma[1,2]. The prevalence of NAFLD is up to 30% in Western countries[3,4] and nearly 5–30% in Asia–Pacific nations[5], making NAFLD the most common cause of elevated liver enzymes and the most common form of liver disease in the world. There are accumulating data showing NAFLD is strongly associated with insulin resistance and other components of metabolic syndrome, such as central obesity, type 2 diabetes, hypertension and hyperlipidemia[6,7]. Because of this strong relationship, NAFLD is regarded as the hepatic manifestation of metabolic syndrome, affecting 30% of the general population[4]. In addition, a 4-year retrospective longitudinal study showed that NAFLD is an independent risk factor of diabetes[8]. The prevalence of NAFLD raises 80–90% in obese adults, 30–50% in diabetic patients and up to 90% in patients with hyperlipidemia. Furthermore, it affects 3–10% of children and up to 40–70% of obese children[9]. Given the high prevalence of NAFLD and its positive correlation with other manifestations of metabolic syndrome, it is important to recognize and aggressively treat this condition. However, there is no satisfying therapeutic strategy for NAFLD. In this perspective, the purpose of the present review was to highlight the available therapies and key molecular modulators for NAFLD tightly associated with metabolic syndrome, especially insulin resistance.

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PATHOGENESIS

Understanding the pathophysiology of NAFLD is still under investigation to develop therapeutic interventions. The most accepted hypothesis is the multi-hits model[10]. The first hit is the accumulation of free fatty acids (FFAs) and TG in hepatocytes, mostly as a result of insulin resistance and obesity. The subsequent hits include a combination of oxidative stress, lipid peroxidation, mitochondrial dysfunction and release of inflammatory mediators, which lead to the progression from steatosis to more advanced stages of liver injury (steatohepatitis and fibrosis). Besides this model, genetic predispositions together with environment factors and metabolic syndrome, such as obesity, diabetes, hypertension and dyslipidemia, are key risk factors for the development and progression of NAFLD[6,7].

Insulin Resistance

Insulin resistance is a physical condition decreasing insulin stimulated glucose uptake in insulin-sensitive tissues, such as muscle and adipose tissue. Furthermore, insulin resistance increases lipolysis in adipose tissue leading to excessive influx of FFAs in the circulation, which directly delivers to the liver. Increased FFAs impair fatty acid oxidation and upregulate *de novo* lipogenesis, all of which contribute to hepatic insulin resistance and fat accumulation[11]. Therefore, insulin resistance is a key factor in hepatic fat deposition, not only due to

hyperinsulinemia, but also due to its association with lipolysis/lipogenesis.

Incretin Hormone

Several clinical studies have shown that glucagon-like peptide-1 (GLP-1)-based treatments ameliorate hepatic steatosis in parallel with obesity and diabetes. In response to a meal, the gastrointestinal hormone, GLP-1, is released from β-cells resulting in incretin effects, such as insulin secretion and reduction of glucagon production[12]. Type 2 diabetes impairs incretin effects, thereby GLP-1-based therapy has been developed for the treatment of diabetes. Exenatide displays biological properties similar to human GLP-1, and is resistant to degradation by dipeptidyl peptidase 4. Recently, both human and obese animal studies have shown that exenatide administration decreases the fat content in the liver by modulating fatty acid oxidation, lipogenesis, insulin secretion from β-cells and hepatic glucose metabolism[13–15]. In our study, we also observed that GLP-1 agonist attenuates high-fat diet-increased hepatic fat levels[16]. Decreased fat accumulation in the liver is associated with exedin 4-mediated glucose and fat metabolism by fatty acid oxidation, sirtulin-related signaling and increased GLP-1 receptor in the liver[17]. Given the favorable effects of GLP-1 agonist on hepatic fat accumulation, the incretin hormone, GLP-1, might be a potential therapeutic target for NAFLD treatment.

Oxidative Stress/Mitochondrial Dysfunction

Numerous experimental and clinical studies have shown a strong link between steatosis and oxidative stress. In patients with NAFLD, there is an increase of oxidative stress-related parameters[18]. Oxidative stress leads to the production of reactive oxygen species, and enhances peroxisomal and mitochondrial β-oxidation, which are increased in patients with NAFLD. Peroxisomal β-oxidation induces the generation of acyl-coenzyme A, which is a major ligand of peroxisome proliferator activator receptor-α (PPAR-α). Highly expressed PPAR-α in the liver senses excess FFAs, increases fatty acid uptake and fatty acid oxidation, lipolysis, and the clearance of lipoproteins. Hepatic steatosis, dyslipidemia and obesity were developed in a PPAR-α deleted animal model[19]. Treatment with fenofibrate, a PPAR-α agonist, in patients with NAFLD improves metabolic syndrome and liver function[20].

Inflammation/Adipokines

Adipose tissue is not only the main site of TG deposition, but also a highly dynamic endocrine organ by secreting several major hormones[21]. Among active peptides produced from adipose tissue, pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), have a critical role in hepatic fat deposition. Serum, and hepatic TNF-α and IL-6 have a positive correlation with the severity of steatosis and fibrosis[22,23]. Theses mediators are strongly associated with central obesity, and have a crucial role in insulin sensitivity, all of which are important in fat accumulation in the liver.

Immune Response

Specific activation of the immune system has been implicated in the pathogenesis of NAFLD. Non-alcoholic fatty liver disease is associated with increased intrahepatic infiltration of natural killer T (NKT) cells, as well as increased levels of pro-inflammatory T helper 1-associated cytokines, TNF-α and IL-6[24,25]. Elevated pro-inflammatory cytokines and hepatic neutrophil infiltration contribute to the development of NASH.

Genetic Polymorphism

The most important genetic polymorphism in the incidence of NAFLD and progression to NASH is patatin-like phospholipase domain-containing 3/adiponutrin, responsible for the TG hydrolysis in adipose tissue. Recent studies have shown that the PNPLA3 genetic variant is strongly associated with hepatic fat accumulation, and the severity of steatosis and fibrosis[26]. There are other reported genetic variants to be correlated with susceptibility of NAFLD, including macrophage migration inhibitory factor[27], adiponectin[28], peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α)[29] and TNF-α[30]. However, more studies to show the conclusive roles of genetic polymorphism in NAFLD are required.

DIAGNOSIS

Early diagnosis and intervention of NAFLD are important due to long-term morbidity and its association with metabolic syndrome. By careful clinical evaluation, it is necessary to rule out personal and family medical history, physical examination and laboratory tests, alcohol consumption, and viral, genetic and autoimmune causes of liver disease before diagnosis[31]. The initial detection of NAFLD is usually based on hepatomegaly and elevated liver transaminases, such as alanine transaminase (ALT) and aspartate transaminase (AST). However, 50% patients show normal liver enzymes, indicating the measurement of liver enzymes are not sensitive for NAFLD diagnosis[32]. Liver biopsy still remains the gold standard to distinguish between simple steatosis and fibrosis, despite the high cost, risk of bleeding, sampling errors and the absence of consensus on pathological interpretation[33]. To overcome biopsy limitation, non-invasive diagnostic assessments, such as computed tomography, magnetic nuclear resonance imaging and proton magnetic resonance spectroscopy, have been developed. These imaging techniques are more sensitive to detection of hepatic steatosis, but are insufficient to determine the stages of disease[34]. Ultrasound-based transient elastography and algorithm-based assays, such as FibroTest, score which combines three variables of body mass index, AST/ALT ratio and the presence of diabetes, NAFLD fibrosis score, and NASH test in combination with clinical and laboratory tests, can be useful for diagnosis[31].

NAFLD MANAGEMENT

The first therapeutic strategy is diet and lifestyle modification. Weight loss, calorie restricted diet, and regular physical exercise

result in a decrease in the incidence of metabolic syndrome and concomitant improvement of hepatic steatosis[35]. However, there is a paucity of data, such as lacking information of long-term outcome, low compliance and frequent regain of bodyweight at follow up, limiting the production of diet and exercise-based guidelines for NAFLD patients. Currently, there is no approved pharmacological treatment for NAFLD. There are possible drug treatments: insulin sensitizers, weight loss medication (rimonabant, inhibitor of cannabinoid [CB1] receptor), lipid-lowering agents (statins, fibrates) and hepatoprotective anti-oxidants (vitamin E, ursodeoxycholic acid, betaine, lipoic acid).

In the association between the endocannabinoid system and appetite, the selective CB1 blocker, rimonabant, was developed and introduced to the market. Indeed, rimonabant results in weight loss by regulating food consumption, lipogenesis and insulin sensitivity[36,37]. In addition to rimonabant mediated weight reduction, CB1 receptor is found in the liver. Therefore, the use of CB1 antagonist improves NAFLD[38]. Statins are well known as lipid lowering drugs by inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase. In addition to their mediated cholesterol synthesis in the liver, statins improve hepatic steatosis, and prevent the development of NAFLD or NASH by their anti-inflammatory and antifibrinogenic actions[39,40]. Another lipid-lowering agent, fibrates, ameliorate NAFLD and improve insulin sensitivity by activating PPAR-α[20]. Among anti-oxidant drugs, vitamin E is the most studied. Vitamin E supplement decreases lipid peroxidation and oxidative stress, which contribute to decreasing the progression for NAFLD to NASH in patients with or without diabetes[41,42]. The mechanisms by which ursodeoxycholic acid, hydrophilic bile acid delays the progression to liver fibrosis and cirrhosis are related to its anti-apoptotic and immunomodulatory effects[43,44]. Betaine, a metabolite of choline, acts as a methyl donor in the homocysteine–methionine cycle and a substitute for S-adenosylmethionine for the direct methylation to phosphatidylcholine[45]. The inhibitory effects of betaine on lipid deposition/infiltration in the liver and liver injury result from its associated phosphatidylcholine production, lipoprotein metabolism and cholesterol transport in the liver[45,46]. A naturally-occurring thiol anti-oxidant, alpha lipoic acid (ALA) acts as an essential cofactor in the citric acid cycle, and is a potent free-radical scavenger. In addition, ALA activates adenosine monophosphate-activated protein kinase (AMPK), and suppresses glucose levels, lipogenesis, insulin resistance and oxidative stress, which in turn decrease liver fat accumulation[47].

Given the close association between metabolic disorder and pathogenesis of NAFLD, pharmacological insulin sensitizing agents are the most promising treatments for NAFLD. Among several insulin sensitizers, the mechanisms, efficacy and usefulness of thiazolidinediones (TZDs) will be extensively discussed in the next paragraph.

Table 1 | Studies testing beneficial the effects of thiazolidinediones on liver fat accumulation in type 2 diabetic patients

Study	Treatment	Participants (*n*)	Length (months)	Liver fat content	Hepatic insulin sensitivity
Mayerson *et al.*[55]	Rosiglitazone	9	3	–38%	ND
Carey *et al.*[56]	Rosiglitazone	33	4	–30%	ND
Bajaj *et al.*[52]	Pioglitazone	14	4	–47%	↑
Sutinen *et al.*[57]	Rosiglitazone	30	6	–15%	ND
Bajaj *et al.*[54]	Pioglitazone	11	4	–48%	↑
Tiikkainen *et al.*[51]	Rosiglitazone	20	4	–51%	↑
Teranishi *et al.*[58]	Pioglitazone	41	6	–30%	ND
Bajaj *et al.*[59]	Pioglitazone	15	3	–50%	↑
Juurinen *et al.*[53]	Rosiglitazone	14	8	–46%	↑

↑, Significant increase after thiazolidinediones treatment; ND, no data. Modified from *Current Opinion in Lipidology*. 2009; 20: 477–83 (66).

Clinical Studies with TZDs

Insulin sensitizers are a potent treatment for NAFLD. Metformin is a widely used first-line drug for type 2 diabetic mellitus. Metformin activates AMPK, leading to a decrease in liver gluconeogenesis, and an increase in muscle glucose uptake and fatty acid oxidation in the adipose tissue[48]. Recently, several research teams[49,50] found that metformin increases blood GLP-1 level, which is involved in insulin secretion and the reduction of glucagon production[12]. As a result, metformin improves peripheral insulin sensitivity. There is another well-known insulin sensitizer, known as TZD. In comparison with metformin, both TZDs (rosiglitazone and pioglitazone) increase peripheral glucose disposal and improve whole-body insulin sensitivity during hyperinsulinemic euglycemic clamp in patients with type 2 diabetes[51,52]. In addition to the favorable effects of TZDs on glycemic control, TZDs decrease fasting FFA concentration and hepatic fat accumulation[53,54]. Table 1 describes the effects of TZDs on hepatic fat accumulation in type 2 diabetic patients. The change of liver fat content was calculated before and after treatment. Therefore, there is no distinct reduction of liver fat level by TZDs in a dose- or time-dependent manner. The decreased rate of hepatic fat accumulation in 4 mg rosiglitazone-treated patients is by 38%[55], and 8 mg treatment is by 30%[56] or 51%[51]; 8 mg rosiglitazone treatment for 4, 6 or 8 months attenuates liver fat content by 30%[56] or 51%[51], 15%[57], or 46%[53], respectively. Pioglitazone treatment (45 mg/day) also ameliorates hepatic fat content in parallel with the increase of insulin sensitivity[52,54,58,59]. Thiazolidinediones-mediated reduction of hepatic fat accumulation has occurred despite weight gain[52–54,56,59]. In addition, several clinical trials have

Table 2 | Studies investigating the role of thiazolidinediones in non-alcoholic steatohepatitis

Study	Treatment	Participants (*n*)	Length (months)	Steatosis	Ballooning/Injury	Inflammation	Fibrosis
Neuschwander-Tetri *et al.*[60]	Rosiglitazone	30	12	↓	↓	NS	NS
Promrat *et al.*[63]	Pioglitazone	18	12	↓	↓	↓	↓
Belfort *et al.*[64]	Pioglitazone	55	6	↓	↓	↓	NS
Lutchman *et al.*[65]	Pioglitazone	18	12	↓	↓	↓	↓
Ratziu *et al.*[61]	Rosiglitazone	63	13	↓	NS	NS	NS
Aithal *et al.*[62]	Rosiglitazone	74	12	↓	↓	NS	NS

↓, Significant decrease after thiazolidinediones treatment; NS, no significance. Modified from *Current Opinion in Lipidology*. 2009; 20: 477–83 (66).

shown that TZDs treatment prevents subsequent events, such as an increase in oxidative stress, lipid peroxidation and pro-inflammatory cytokines that contribute to the development of NAFLD to NASH. Table 2 shows that the treatment of TZDs for NASH improves metabolic and histological predictors. Rosiglitazone[60–62] and pioglitazone[63–65] treatments reduce liver transaminotransferase levels, hepatic fat content and histological features mostly in regard to steatosis, ballooning and liver injury. From table 1 and 2[66], we summarize that TZDs treatment attenuates not only liver fat content, but also histological lesions. Still, larger extended trials to determine long-term TZDs treatment prevents liver injury and the progression to NASH are required.

Adverse Effects of TZDs in Clinical Practice

Several unfavorable effects of TZDs have been observed. The first-introduced TZD, troglitazone, was associated with severe hepatotoxic side-effects, leading to withdrawal from the market[67]. However, other agents in the same class of glitazones, rosiglitazone and pioglitazone, did not induce any symptoms of hepatic dysfunction[68,69]. Edema is one of the most frequent side-effects of TZDs. From a meta-analysis study, Berlie *et al.*[70] found the positive association between TZDs and development of edema. Thiazolidinediones increased edema results from renal excretion of sodium and intestinal ion transport, which raise plasma volume/fluid retention[71,72]. However, the severity of edema by TZDs is usually trivial, and TZDs-induced edema

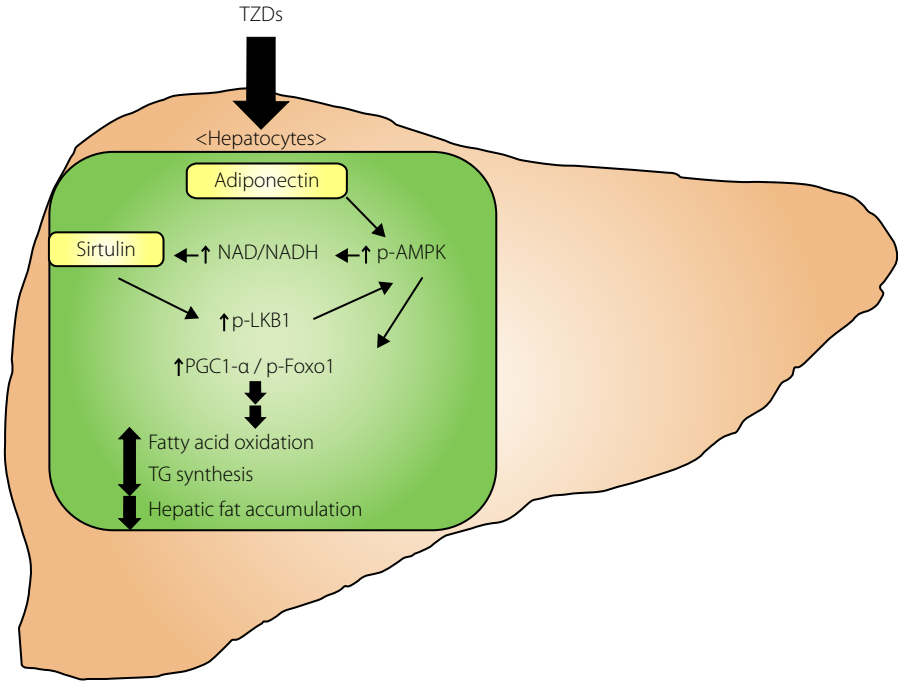

Figure 1 | Proposed potential mechanisms by which the treatment of thiazolidinediones (TZDs) improves hepatic steatosis. In the liver, TZDs upregulate adiponectin and/or sirtulin, which consequently alter hepatic regulators, leading to an increase in fatty acid oxidation and decrease in fat accumulation. AMPK, adenosine monophosphate-activated protein kinase; Foxo1, forkhead box O1; LKB1, liver kinase B1 also known as serine/threonine kinase 11; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; PGC-1α, peroxisome proliferator-activated receptor gamma coactivtor-1α.

might be prevented with the use of diuretics. There is a strong positive association between TZDs and heart failure. From teleo-analysis, the use of TZDs for 2.2 years will develop one incidence of heart failure in every 50 patients[73]. Based on this correlation, the American Heart Association and American Diabetes Association made a statement that TZDs should not be prescribed to patients with a high risk of heart disease[74]. Furthermore, TZDs treatment decreases bone density and increases the risk of fractures by increasing adipogenesis in bone marrow and weight gain[75,76]. Increased bodyweight is a risk factor of diabetes; however, TZDs-induced weight gain is not correlated with decreased insulin sensitivity[77]. This increase of weight is accompanied by desirable fat distribution and accumulation in subcutaneous adipose tissue[78,79].

Mechanisms of TZDs Action

Peroxisome proliferator-activated receptor γ (PPARγ) agonists, TZDs, have beneficial effects on insulin sensitivity by regulating the transcription of several genes in glucose and lipid metabolism[80,81]. Thiazolidinediones decrease lipolysis, promote fatty acid uptake and storage in adipose tissue, leading to an increase in adipose tissue mass[55,82]. Thiazolidinediones-induced fatty acid uptake and storage in adipose tissue prevent other insulin-sensitive tissues, especially the liver, from deleterious metabolic effects of FFA. In addition, TZDs administration affects the production of adipokines. Thiazolidinediones decrease pro-inflammatory cytokine, TNF-α, which is positively associated with the degree of steatosis and fibrosis[22,23,83]. An anti-inflammatory adipokine, adiponectin, is also increased by TZDs in both type 2 diabetic animals and humans[84,85]. Adiponectin stimulates fatty acid oxidation by activating AMPK, and inhibits lipid accumulation by modulating acetyl coenzyme A carboxylase and PGC-1α[86,87]. Therefore, there is a possibility that adiponectin is one of the key mediators in TZDs-decreased hepatic fat accumulation.

Our group suggests sirtulin as a crucial candidate of TZDs-decreased NAFLD. The mammalian sirtuins (silent information regulator 2 proteins) are a family of nicotinamide adenine dinucleotide-dependent deacetylases and adenosine diphosphate-ribosyltransferase[88]. Recent studies have shown that sirtulin 6 (SIRT6) plays an important role in glucose and lipid metabolism. Liver-specific ablation of SIRT6 in mice regulates gene expression, which consequently increases glycolysis, TG synthesis and decreases fatty acid oxidation. Thereby, liver-specific SIRT6 deletion results in fatty liver formation[89]. Furthermore, SIRT6 prevents the progression to NASH by regulating pro-inflammatory cytokines, such as IL-6 and TNF-α[90]. In our study[91], we highlight the role of TZDs-induced SIRT6 in hepatic fat deposition. Rosiglitazone administration decreases hepatic TG accumulation, and concomitantly enhances gene expression of adiponectin and SIRT6, together with changes in key mediators of fatty acid oxidation, such as PGC-1α, forkhead box O1 (Foxo1), phosphorylation of AMPK and liver kinase B1 (LKB-1). Consistent with previous studies, SIRT6 deletion by ribonucleic acid interference-mediated gene silencing in hepatocytes leads to hepatocyte fat accumulation accompanied by alterations in messenger ribonucleic acid and protein expression of PGC-1α and Foxo1 and phosphorylation levels of LKB1 and AMPK, which are closely related to fatty acid oxidation. Therefore, we propose the possible mechanisms by which TZDs ameliorate fat accumulation in the liver are involved in TZDs-induced adiponectin and/or SIRT6 (Figure 1). SIRT6 might serve as a therapeutic target for NAFLD[92].

CONCLUSIONS

Hepatic steatosis is a result of complex interplay between diet, the metabolic system and major tissues, such as adipose tissue and the liver, but a full understanding of its pathogenesis has not yet been determined. Thereby, no drug is available as a specific treatment for NAFLD. Given the association between metabolic syndrome and hepatic fat accumulation, a well-known insulin sensitizer, TZDs, has been of interest to researchers looking for a potential treatment. Indeed, TZDs play a crucial role in metabolic alterations associated with NAFLD by targeting several different genes, including adiponectin and sirtulin, in the liver. In regard to TZDs-induced safety concerns and efficacy, several options can be considered to improve the benefit-to-risk ratio of PPARγ-modulating drugs. Selective PPARγ modulators, such as MBX-102 and INT131, have a high affinity interaction with PPARγ. A new synthetic PPARγ, SR1664, blocks cyclin-dependent kinase 5-mediated phosphorylation of PPARγ without transcriptional change. Notably, translating the novel therapeutic potential observed in animal studies to humans, and long-term effects, will remain challenging.

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