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Glitazones for human nonalcoholic steatohepatitis

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***Abstract*:** The rationale for specific pharmacologic therapy in nonalcoholic steatohepatitis (NASH) is determined by the potential for disease progression and the difficulties, in many patients, of successfully implementing diet and lifestyle changes over the long term. Owing to their ability to correct insulin resistance, insulin-sensitizing agents are attractive candidates for the treatment of NASH. In this review we provide an insight into the mechanism of action, therapeutic efficacy and safety issues regarding the use of glitazones in NASH.

***Keywords*:** nonalcoholic steatohepatitis, steatosis, fibrosis, insulin resistance, glitazones, insulin sensitizing drugs

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Introduction

Nonalcoholic steatohepatitis (NASH) is becoming the leading cause of chronic liver disease and a major health issue owing to its close association with the worldwide epidemics of obesity and diabetes. A significant proportion of patients can experience disease progression with the occurrence of cirrhosis, hepatocellular carcinoma [Ascha *et al*. 2010; Starley *et al*. 2010; Paradis *et al*. 2009; Bugianesi *et al*. 2002; Ratziu *et al*. 2002] and end-stage liver disease [Nayak *et al*. 2010; Caldwell and Crespo, 2004]. This results in an increase in the overall and liver-related mortality [Soderberg *et al*. 2010; Gastaldelli *et al*. 2009b; Ong *et al*. 2008; dam-Larsen *et al*. 2004]. Patients at risk of disease progression need to be identified as not all individuals with metabolic risk factors will experience disease progression [Ratziu *et al*. 2010a]. Prognostic markers have mostly been derived from histological studies and found that the degree of inflammation is the strongest independent predictor for fibrosis progression [Argo *et al*. 2009].

Insulin resistance is an almost universal finding in primary NASH. It is the main driving force behind excessive fat accumulation in the liver but may also play a role in the initiation and perpetuation of steatohepatitis and fibrosis progression [Fabbrini *et al*. 2009; Fracanzani *et al*. 2008; Marchesini *et al*. 2003]. Moreover, hepatic steatosis and insulin resistance potentiate each other [Malhi and Gores, 2008; Yamaguchi *et al*. 2007; Bugianesi *et al*. 2005a]. A current model for the pathogenesis of NASH is centered on lipotoxicity [Neuschwander-Tetri, 2010a] which states that the influx of fatty acids and their derivatives through the liver induces apoptosis, oxidative stress, reticulum endoplasmic stress, activation of proinflammatroy pathways and ultimately liver cell injury. The main source of free fatty acids (FFA) reaching the liver is an uncontrolled release from insulin-resistant adipose tissue [Cusi, 2009; Petta *et al*. 2009]. Therefore, correcting insulin resistance, particularly at the adipose level, is a relevant aim and most therapeutic trials have focused on insulin sensitizers [Ratziu and Zelber-Sagi, 2009].

The aim of this report is to review the existing trials with glitazones in NASH with a focus on their histological, biochemical and metabolic effects.

Mechanism of action

Glitazones are agonists of PPARγ (peroxisome proliferators-activated receptor gamma) nuclear receptors, developed for the treatment of type 2 diabetes and which have been on the market for almost a decade.

The PPARγ is a member of the nuclear protein receptor superfamily which regulates the transcription of genes involved in lipid metabolism and plays a role in increasing insulin sensitivity as well as in promoting fatty acid uptake into

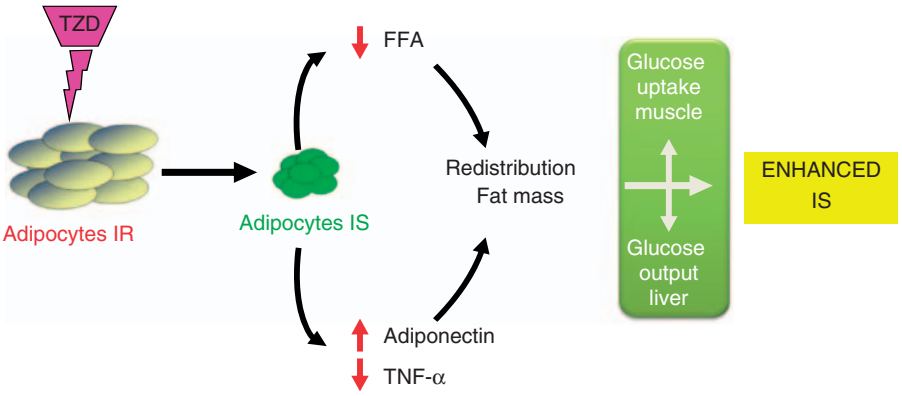

Figure 1. Mechanism of action of glitazones. Glitazones promote the differentiation of small, metabolically active, insulin-sensitive (IS) adipocytes from large, insulin-resistant (IR) adipocytes. This results in decreased free fatty acids (FFA) influx to the liver, decreased tumor necrosis factor alpha (TNFα) expression, increased adiponectin production and redistribution of fat mass. The consequence is an increase in the storage of fatty acids in adipose tissue, a reduction of hepatic glucose production and a higher uptake of the glucose in the muscles which results in enhanced insulin sensitivity.

adipocytes and adipocyte differentiation [Sharma and Staels, 2007]. PPARγ receptors are located predominantly in adipose tissue, but can be found elsewhere, to include pancreatic β cells, vascular endothelium and, to a lesser extent, in liver and skeletal muscle. Two isoforms are recognized, PPARγ1 and the less abundant PPARγ2. Obese patients have an increased expression of PPARγ2 in the adipose tissue [Sharma and Staels, 2007].

Glitazones promote the differentiation of large, insulin-resistant adipocytes into small, metabolically active, insulin-sensitive adipocytes (Figure 1). Data suggest that thiazolidinediones (TZDs) decrease FFA influx to the liver, decrease tumor necrosis factor alpha (TNFα) and resistin expression and increase adiponectin production [Gastaldelli *et al.* 2009a]. Increased adiponectine expression results in reduced hepatic gluconeogenesis and improved hepatic fatty acid oxidation (via increased AMP-activated protein kinase [AMPK]). Adiponectine also reduces inflammation by blocking nuclear factor κB (NFkB) and suppress hepatic stellate cell proliferation [Bugianesi *et al.* 2005b]. The net result is an increase in the storage of fatty acids in adipose tissue, a reduction of hepatic glucose production and a higher uptake of the glucose in the muscles. This redistribution of fat from ectopic tissue (liver, muscle) to the adipose tissue is probably the main determinant of the insulin-sensitizing action of this class of drugs.

Recent evidences suggest that there also are differences in lipoprotein metabolism between the two TZDs (rosiglitazone and pioglitazone), attributable to an additional PPARα activity of pioglitazone, not shared by rosiglitazone. This results in increased fatty acid oxidation and decreased hepatic *de novo* lipogenesis, which may explain, at least in part, the positive cardiovascular effects (improved carotid intimal medial thickness and coronary atheroma volume) with pioglitazone [Nissen *et al.* 2008; Betteridge, 2007; Mazzone *et al.* 2006].

Efficacy of glitazones in human adult NASH

Both TZDs have been studied in prospective placebo-controlled trials ranging from 6 to 24 months duration. Here we discuss trials of pioglitazone and rosiglitazone, in patients with histologically proven NASH and an end-of-treatment liver biopsy. Seven trials were selected: five randomized controlled trials (RCTs) [Sanyal *et al.* 2010, 2004; Aithal *et al.* 2008; Ratziu *et al.* 2008; Belfort *et al.* 2006] and two open-label trials [Promrat *et al.* 2004; Neuschwander-Tetri *et al.* 2003] (Table 1).

Biochemical response

One of the most reproducible effects of glitazones is a reduction of aminotransferase levels by 30–58%. This occurs early on with therapy (starting at month 4 and almost complete by month 6) and is sustained throughout the treatment period. ALT normalization was reported in

Table 1. Glitazone trials with available end-of-treatment histology.

Trial	Drug (dose/day), *n*	Comparator, *n*	Treatment duration	End of treatment histology, *n* (drug/ comparator)	Percentage with diabetes	Normal ALT included	Professional diet counseling*	Run-in period
Randomized controlled trials								
Sanyal *et al.* 2004]	Pioglitazone (30 mg) + Vit E, *N* = 10	Vit E, *N* = 10	6 months	18 (9/9)	0%	Yes	Yes	Yes (no results)
Belfort *et al.* [2006]	Pioglitazone (45 mg) *N* = 29	Placebo *N* = 24 total 55)	6 months	47 (26/21)	100%	No	Yes	Yes
Ratziu *et al.* [2008]	Rosiglitazone (8 mg), *N* = 32	Placebo, *N* = 32	1 year	63 (32/31)	33%	No	No	No
Aithal *et al.* [2008]	Pioglitazone (30 mg), *N* = 37	Placebo, *N* = 37	1 year	61 (31/30)	0%	Yes	No	Yes (no results)
Sanyal *et al.* [2010]	Pioglitazone (30 mg), *N* = 80	Placebo, *N* = 84,	2 years	142 (70/72)	0%	Yes	No	No
Open label trials								
Neuschwander-Tetri *et al.* [2003]	Rosiglitazone (8 mg), *N* = 25	None	12 months	22	24%	Yes	No	No
Promrat *et al.* [2004]	Pioglitazone (30 mg), *N* = 18	None	1 year	18	0%	No	No	Yes

*That is, follow-up visits with a dietician.
Vit, vitamin; ALT, alanine transaminase.
Adapted from Ratziu *et al.* [2010b] and Ratziu and Zelber-Sagi [2009], with permission.

38–100% of patients. This effect is short lived, since after drug discontinuation a return to baseline levels usually occurs within 3 months [Ratziu *et al*. 2008]. Longer treatment (>1 year) does not seem to have additional beneficial effects [Sanyal *et al*. 2010; Ratziu *et al*. 2010c]. However, reported trials are heterogeneous in terms of baseline ALT. Patients with normal ALT have been included in some of them [Sanyal *et al*. 2010, 2004; Aithal *et al*. 2008] (three of five RCTs) which could reduce the magnitude of the biochemical effect.

Histological response

Steatosis. Steatosis is the histologic feature most reliably improved by glitazones and was reported in all except one trial [Aithal *et al*. 2008]. In the one negative trial, 26% of patients had minimal steatosis at baseline (5–25%), making differences between groups harder to detect [Aithal *et al*. 2008]. An improvement in steatosis was reported in 47–65% of treated patients, but the magnitude of this effect was reported in only a few trials. With rosiglitazone it was 20% ranging from 30% to 60% in responders [Ratziu *et al*. 2008].

Necroinflammatory activity. The impact of glitazones on inflammation, ballooning and fibrosis is summarized in Table 2. Improvement of inflammation is of particular importance, since a recent study found that inflammation was the only independent predictor for fibrosis progression [Argo *et al*. 2009]. In RCTs, regression of inflammatory lesions was statistically significant only in two trials with pioglitazone [Sanyal *et al*. 2010; Belfort *et al*. 2006]. In one study using a lower dose of pioglitazone (30 mg/day), the improvement *versus* placebo was not significant after 1 year of therapy [Aithal *et al*. 2008]. The only RCT with rosiglitazone did not find significant changes in lobular inflammation after 1 or 2 years of therapy [Ratziu *et al*. 2010c, 2008]. Portal inflammation was either unchanged or worsened in one study with rosiglitazone [Neuschwander-Tetri *et al*. 2003].

Ballooning improved in 32–54% of patients which was significantly more than placebo in two RCTs with pioglitazone [Aithal *et al*. 2008; Belfort *et al*. 2006]. Rosiglitazone improved ballooning in a small uncontrolled trial [Neuschwander-Tetri *et al*. 2003], but no significant improvement was found in a large RCT trial after 1 or 2 years of therapy [Ratziu *et al*. 2010c, 2008]. Very few studies have reported on changes in the recently described NAS score. In a 6-month study [Belfort *et al*. 2006], the score improved in 46% of pioglitazone-treated patients by at least 2 points *versus* 14% in the placebo group ($p = 0.02$) although this could be caused by the improvement in steatosis that is a part of the score. A 1-year study with rosiglitazone failed to show significant changes in the NAS score [Ratziu *et al*. 2008] while a 2-year study with pioglitazone improved the NAS score significantly more often than placebo [Sanyal *et al*. 2010]. In this latter trial [Sanyal *et al*. 2010] pioglitazone failed to reach the primary endpoint, a complex composite score, at a predefined level of statistical significance of 0.025. However, the negative results of this study on the primary endpoint should be regarded with caution, because 28% of patients in the pioglitazone arm did not have ballooning on the central pathological review performed at the time of final analysis. Thus, it can be argued that some of the included patients did not have sufficient histological criteria for NASH. Further sensitivity analyses have shown that in the subset of patients with well-defined NASH, pioglitazone improved histology (except for fibrosis) including the composite endpoint more often than placebo.

Fibrosis. No study conclusively demonstrated an improvement in fibrosis. In three trials, including an uncontrolled one, improvement of fibrosis was seen in 29–61% of patients taking pioglitazone [Aithal *et al*. 2008; Belfort *et al*. 2006; Promrat *et al*. 2004]. Despite an end of treatment reduction, when compared with changes in placebo/control arms, improvement of fibrosis was still significant in only one study with a marginal level of statistical significance. No significant improvement of fibrosis was seen with rosiglitazone [Ratziu *et al*. 2010c, 2008] even after prolonged therapy, and when measured by micromorphometry, a more sensitive and quantitative technique. Thus, it becomes improbable that the lack of effect seen after 1 year could be due to the short treatment period. Only two studies assessed changes in perisinusoidal fibrosis, one documenting no change [Ratziu *et al*. 2008] and the other an improvement in 35% [Neuschwander-Tetri *et al*. 2003].

Overall, data available so far show that the insulin-sensitizing effect of glitazones is associated with a significant improvement of aminotransferases and steatosis, most probably of inflammation

Table 2. Outcomes of glitazone studies for inflammation, liver cell injury and fibrosis.

Trial	Drug	Dose/duration	LOBULAR INFLAMMATION		BALLOONING		FIBROSIS	
			Intragroup change (trend, *p*)	Change *vs.* comparator (trend, *p*)	Intragroup change (trend, *p*)	Change vs. Comparator (trend, *p*)	Intragroup change (trend, *p*)	Change *vs.* Comparator (trend, *p*)
Neuschwander-Tetri *et al.* [2003]	Rosiglitazone	8 mg/12 months	–	–	I, 0.003	–	I-PSF	–
Promrat *et al.* [2004]	Pioglitazone	45 mg/12 months	I, <0.001	–	I, 0.001	–	I, 0.04	–
Sanyal *et al.* [2004]	Pioglitazone	30 mg/6 months	–	–	I, 0.01	NC	NC	NC
Belfort *et al.* [2006]	Pioglitazone	45 mg/6 months	I, <0.001	I, 0.008	I, 0.001	I, <0.02	I, 0.002	NC, 0.08
Ratziu *et al.* [2008]	Rosiglitazone	8 mg/12 months	NC	NC	NC	NC	NC	NC
Aithal *et al.* [2008]	Pioglitazone	30 mg/12 months	I, 0.04	NC	NC, 0.09	I, 0.005	I, 0.006	I, 0.05
Sanyal *et al.* [2010]	Pioglitazone	30 mg/2 years	I, –	I, <0.001	I,–	I, 0.01	NC	NC

I, improved; NC no change -, not available; PSF, Zone 3 perisinusoidal fibrosis.
Adapted from Ratziu *et al.* [2010b], with permission.

but no effect on fibrosis [Ratziu *et al.* 2010b]. It is less likely that these results are influenced by sampling variability of liver biopsy, since in controlled trials sampling variability should affect the active treatment and placebo arms equally. Reduction in liver fat has important clinical implications, both hepatic and extrahepatic. Steatosis is the prerequisite in the sequential multistep process that leads to NASH and its progression. Steatosis is the *trigger* for lipid peroxidation and oxidative stress which contributes to the necroinflammatory lesions associated with steatohepatitis. It is also associated with increased hepatic expression of proinflammatory cytokines and mitochondrial dysfunction which results in increased apoptosis and decreased energy stores [Neuschwander-Tetri, 2010b]. Moreover, hepatic stellate cell activation by reactive oxygen species and lipid peroxidation products is an essential trigger for fibrogenesis [Feldstein *et al.* 2005]. Extrahepatic consequences of liver fat are also important since steatosis *per se* might aggravate hepatic insulin resistance independently of central fat. However, this beneficial effect of reduction in liver fat has been challenged by some animal studies showing that liver fat is not all bad. Blocking esterification of fatty acids into triglycerides resulted in a higher level of hepatic oxidative stress, inflammation, cell injury and fibrosis in animals fed a methionine choline-deficient (MCD) diet [Yamaguchi *et al.* 2007]. These observations suggest that triglycerides synthesis *per se* is not harmful to hepatocytes, but it rather provides a useful mechanism for protecting the liver from lipotoxicity.

Assessing the durability of the histological response to glitazone therapy is difficult since no histological follow up is available except for a series of nine pioglitazone-treated patients [Lutchman *et al.* 2007]. Histological criteria of steatohepatitis (steatosis, lobular inflammation, ballooning), which disappeared in all but one patient after 1 year of pioglitazone therapy, relapsed in most of the patients 1 year after treatment discontinuation. There was no worsening in fibrosis with this short follow up. Similarly, in the open-label FLIRT-2 trial, an additional 2 years of treatment with rosiglitazone did not further improve liver histology, despite a continued improvement in insulin

sensitivity and aminotransferases [Ratziu *et al.* 2010c].

Metabolic response

Changes in insulin resistance were assessed by clamp studies or surrogate markers such as hyperinsulinemia or the HOMA index in both diabetic [Ratziu *et al.* 2008; Belfort *et al.* 2006] and nondiabetic patients [Sanyal *et al.* 2010, 2004; Promrat *et al.* 2004].

In diabetic patients, both TZDs induced a decrease in insulin levels of 30–34%, as well as a significant reduction in serum glucose and HOMA [Ratziu *et al.* 2008; Belfort *et al.* 2006]. Moreover a 1.9- to 2.8-fold increase in adiponectin was documented with both glitazones. Changes in serum adiponectin levels correlated negatively with a reduction in steatosis [Ratziu *et al.* 2008; Lutchman *et al.* 2006]. It has been shown that the increase in adiponectin levels and the improvement in insulin resistance with glitazones are causally related through hepatic effects [Gastaldelli *et al.* 2009a], namely a decrease in hepatic glucose production and an increase in hepatic adenosine monophosphate-activated protein kinase. Importantly, improvement in insulin sensitivity was well correlated with a reduction in liver fat and aminotransferase values but not in necroinflammatory lesions. After drug discontinuation, aminotransferases serum insulin and HOMA were still maintained at 3 months [Ratziu *et al.* 2008] but rose above baseline 1 year after, while adiponectin declined [Lutchman *et al.* 2007].

Interestingly, the decline in HOMA index was significantly higher in patients with steatosis reduction (93%) although 59% of patients with unchanged steatosis also experienced a reduction in HOMA [Ratziu *et al.* 2008]. These findings suggest that correcting insulin resistance is necessary but not sufficient for treating NASH and that, at least in some patients, different pathways (such as inflammatory or fibrotic cascades) should be targeted.

Adverse effects of glitazones

The main drawback of using glitazones is their safety profile. Therefore, potential hepatic benefit of this class of drugs needs to be weighed against long-term safety issues, of particular concern being cardiovascular toxicity, osteoporosis and weight gain.

Glitazones and cardiovascular toxicity

Significant concerns have been raised about the potential of both glitazones to increase cardiovascular morbidity and related mortality. Cardiovascular risk appears to be drug related rather than a class effect, and seemed to be higher with rosiglitazone than pioglitazone.

Concerns about adverse cardiovascular effects were first raised in 2007, when a meta-analysis of data from 42 clinical trials found a significant increase in the relative risk of myocardial infarction (odds ratio [OR] = 1.43, 95% confidence interval [CI], 1.03–1.98; $p = 0.03$), and of death from cardiovascular causes (OR = 1.64 95% CI, 0.98–2.74; $p = 0.06$), among type 2 diabetics treated with rosiglitazone [Nissen and Wolski, 2007].

Following this meta-analysis an unplanned interim analysis of RECORD trial, the largest, randomized, long-term trial of the cardiovascular safety of rosiglitazone compared with other drug interventions for type 2 diabetes, was performed [Home *et al.* 2007]. This analysis was inconclusive, with data being insufficient to determine whether the drug was associated with an increase in the risk of myocardial infarction or death from cardiovascular causes. The finals results of RECORD trials published in 2009 are inconclusive about effects on myocardial infarction, and conclude that rosiglitazone increase the risk of heart failure but does not increase the risk of overall cardiovascular morbidity or mortality compared with standard glucose-lowering drugs [Home *et al.* 2009]. The RECORD trial has certain built-in limitations, particularly its open-label design, its relatively small size (for a cardiovascular trial) and the choice of the primary endpoint. Two longer-term, double-blind RCTs of rosiglitazone (DREAM and ADOPT) were completed around the time of the original meta-analysis. These trials did not show an increased mortality, but had numerically (not statistically significant) higher rates of myocardial infarction in the rosiglitazone arms [Gerstein *et al.* 2006; Kahn *et al.* 2006].

An update of the 2007 US Food and Drug Administration (FDA) meta-analysis, including 52 trials, was performed in 2010 and supported the original concern that rosiglitazone increases the risk of heart attacks, and thereby might increase the risk of cardiovascular death and all-cause death, when compared with placebo or

non-TZD diabetes drugs. In addition, the vast majority of these events in the meta-analysis come from trials of 12 months duration or less. Therefore, the hypothesis raised by this meta-analysis is that the risk of myocardial infarction, and potentially other serious cardiovascular events, occurs promptly after exposure to rosiglitazone, during the first year of therapy.

Recently the FDA decided to further restrict the use of rosiglitazone in the US, and to continue the ongoing cardiovascular safety trial, called TIDE (Thiazolidinedione Intervention with Vitamin D Evaluation), to compare rosiglitazone to other diabetes treatments such as pioglitazone. GSK was asked to perform a re-adjudication of the RECORD study. In Europe the European Medicines Agency (EMEA) decided to suspend the marketing authorization of rosiglitazone following a review by the Committee for Medicinal Products for Human Use (CHMP), initiated on 9 July 2010.

Unlike rosiglitazone, pioglitazone has not been associated with increased risks of cardiovascular events or mortality. The prospective, placebo-controlled PROactive trial of 5238 subjects with diabetes and known cardiovascular disease demonstrated no increase in risk of all-cause mortality, nonfatal myocardial infarction and stroke in patients with type 2 diabetes who have a high risk of macrovascular events [Dormandy *et al.* 2005]. This was confirmed by a subsequent meta-analysis [Lincoff *et al.* 2007]. The risk of congestive heart failure (CHF) in PROactive trial, 38% over the entire treatment period, was significantly higher in the pioglitazone group but the mortality rate from heart failure was not different in the placebo and the pioglitazone arms [Dormandy *et al.* 2005]. The only predictive factors for severe heart failure were older age >65 years and obesity [Lincoff *et al.* 2007]. The current recommendations are to avoid the use of pioglitazone in patients with severe heart failure.

Weight gain

Weight gain is a well-recognized side effect of glitazones mainly because of expansion of peripheral and subcutaneous adipose tissue. The weight gain with glitazones is associated with an increase in peripheral adipose tissue and a concomitant decrease in visceral fat content. This fat redistribution is explained by PPARγ agonist-induced remodeling of abdominal fat tissue, characterized by differentiation of preadipocytes into small fat cells in subcutaneous fat depots and apoptosis of differentiated large adipocytes (hypertrophic adipocytes) in visceral and/or subcutaneous fat depots. Fat is thus cleared from muscle and liver and redirected into inert storage sites, which could explain why glitazones improve adipose tissue insulin resistance and glucose metabolism despite weight gain [Miyazaki *et al.* 2002]. This increase in body weight is not associated with an increased cardiometabolic risk.

Weight gain most frequently develops within the first few months of treatment and appears to plateau thereafter, although there can be additional weight gain over time. In the largest pioglitazone trial, a significant weight gain was noted by week 24 and progressed over the course of the study, with a mean of 4.7 kg at week 96. In rosiglitazone trials, a weight gain of more than 3 kg was noted in 30% and 36% of the patients after one and two additional years of treatment, respectively [Ratziu *et al.* 2010c, 2008].

Bone loss and fractures

Recently, clinical studies have confirmed that TZDs can damage the bones although the confounding adverse effect of diabetes *per se* cannot be ruled out. Clinical studies demonstrated a decreased cortical bone mass in hands and feet in patients with type 1 diabetes mellitus. Studies in rodent models and humans indicate that glitazones impairs osteoblastic function, resulting in reduced bone formation and bone mass but do not affect bone resorption *in vivo* [Grey, 2008].

A *post hoc* analysis of ADOPT trial [Kahn *et al.* 2006] reported a significantly higher incidence of fractures in the appendicular skeleton in women but not in men. A recent meta-analysis confirmed that overall, use of glitazones significantly increase the risk of fracture in women but not in men and is also associated with significant changes in bone mineral density at the lumbar spine and hip [Loke *et al.* 2009].

Conclusions

By correcting insulin resistance, glitazones are logical drug candidates for the treatment of NASH. Although imperfect, the existing glitazones studies highlight the methodological challenges for future clinical trials. Whether the partial efficacy of glitazones on both biochemical and histological outcomes can be improved by better selection of patients and better identification of predictors of response needs to be

further determined. Given the complexity of mechanisms involved in the progression of the disease, simply correcting insulin resistance will not be enough for a majority of patients. Combining insulin-sensitizing agents with hepatoprotective or anti-inflammatory/antifibrotic drugs in nonresponders or partial responders is a very attractive option for future therapeutic strategies.

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Conflict of interest statement

VR has worked as an occasional consultant regarding NAFLD projects for Axcan Pharma, Astellas, Genentech, Gilead, Pfizer, Roche, Sanofi-Aventis.

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