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Classical and Innovative Insulin Sensitizing Drugs for the Prevention and Treatment of NAFLD

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Abstract: *Background*. Nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disorder worldwide, comprises a spectrum of conditions ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. NASH is associated with an increased risk of hepatocellular carcinoma (HCC) and cardiometabolic disease. Insulin resistance (IR) is the underlying pathogenic mechanism for NAFLD, the presence of which in turn, is a strong predictor for the development of metabolic disorders. Hence, therapy of NAFLD with insulin-sensitizing drugs (ISDs) should ideally improve the key hepatic histological changes (steatosis, inflammation and fibrosis), but should also reduce cardiometabolic and cancer risk.

Objectives. In this review, the rationale for the use of ISDs and the evidence for their efficacy are detailed. In particular, the mechanism of action, potential for use, limitations and untoward effects of metformin and thiazolidinediones are systematically reviewed. Further, we discuss novel ISDs that may have potential clinical utility in NAFLD.

Results and Conclusion. Despite the theoretical prediction that ISDs might have beneficial effects on disease outcomes, evidence that ISDs are able to alter the natural history of NAFLD are presently not available. The exploration of novel strategies exploiting "non-conventional" ISDs is encouraged.

Keywords: Adiponectin; dipeptidyl peptidase-IV inhibitors; estrogens; glucagon-like peptide-1; hepatocellular carcinoma; insulin sensitizing drugs; metformin; nonalcoholic fatty liver disease; rimonabant; thiazolidinediones; ursodeoxycholic acid.

BACKGROUND

Nonalcoholic Fatty Liver Disease (NAFLD), a spectrum of disorders ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) with/without cirrhosis, and hepatocellular carcinoma (HCC) [1] is the most common chronic liver disease, with a prevalence of 25% in the general population of Western Countries [2].

The prevalence of NAFLD is increased in subjects with type 2 diabetes mellitus (35%-90%), obesity (10%-80%), hypertension (30%-56%) and dyslipidemia (26%-58%)[3]. Age, gender and ethnicity also influence NAFLD prevalence; NAFLD is more common in older subjects and in males, but prevalence rates could be an underestimate given the often suboptimal diagnostic methods used [4]. The prevalence of hepatic steatosis in the Dallas Heart Study followed a clear-cut gradient with Hispanics being more affected than whites, and African Americans ranking as the ethnic group with the least prevalence of NAFLD [5]. A more recent analysis confirmed that ethnicity represents a major determinant for the risk of developing NASH for a given level of insulin resistance (IR) [6]. Primary NAFLD is typically associated with IR and the Metabolic Syndrome (MetS) [7]. Diagnosis requires the exclusion of other causes of liver disease such as viral infection, autoimmune diseases, alcohol abuse, endocrine and genetic disorders [8].

NAFLD is an early predictor of metabolic disorders, even in non-diabetic and non-obese subjects [9]. It is associated with an increased risk for all cause mortality, end-stage liver disease, cardiovascular disease, HCC and some hepatic and extrahepatic cancers [10,12-20]. Despite the relative accuracy of non-invasive methods for predicting advanced fibrosis [10], liver biopsy remains the gold-standard for the grading and staging of NAFLD [2,11]. In animal studies, a direct progression from steatosis to NASH has been observed, leading to speculation that "multiple hits" are involved in the pathophysiology of a single disease spanning a wide spectrum . However, more recent views support the concept that simple steatosis and NASH appear as two distinct entities and progression from pure fatty liver to NASH appears to be so rare as to warrant publication [84]

The ideal treatment for NAFLD should improve the key histological changes of NASH (steatosis, inflammation and fibrosis) and reduce morbidity and mortality from cardiometabolic diseases and cancer, particularly HCC [14,21]. Diabetic patients are known to have a high prevalence of NAFLD/NASH [22] and are recognized to be at higher risk for HCC [23-30]. Additionally, in diabetics, 50% of cases of HCC develop in non-cirrhotic livers [31-33] and overall, only 46% of patients with NAFLD and HCC have been shown to have cirrhosis [34]. The putative mechanisms underlying the development of HCC in NAFLD relate to IR and to the associated inflammatory cascade [35]. Hence, Insulin-sensitizing drugs (ISDs) might be effective in preventing NAFLD-associated HCC, but data to confirm or refute this assertion are still awaited.

ISDs therapy of NASH has been extensively investigated. However, most trials are relatively small, proof-of-concept studies. The largest body of data in humans is available for two classes of ISDs: thiazolidinediones (TZDs) and biguanides.

In the present review, the rationale for the use, and data on the efficacy for both ISD classes will be detailed. Next, we discuss more innovative therapies that, acting through insulin sensitization, hold promise for clinical utility in NAFLD and therefore need to be validated by testing in the clinical arena.

SECTION 1. SHOULD WE TREAT INSULIN RESISTANCE TO CURE NAFLD?

A). Pathogenesis of IR

IR may be defined in multiple ways but eventually results in impaired response to insulin actions. The pathogenesis of IR resides

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in imbalanced energy homeostasis leading to ectopic lipid accumulation [36]. Physical inactivity, excess caloric intake and altered dietary composition are key exogenous factors that promote the development of a fatty liver which, in turn, promotes IR [37-43].

At the molecular level, hyperglycemia associated with hyperinsulinemia promotes the development of hepatic steatosis via up-regulation of the lipogenic transcription factors including sterol regulatory element binding protein-1 (SREBP-1) and peroxisome proliferator-activated receptor gamma (PPAR-γ), and their downstream effector enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) [44].

In the adipose tissue, impaired insulin-mediated inhibition of hormone-sensitive lipase is conducive to increased circulating levels of free fatty acids (FFA) [45], which also closely correlate with liver fat content and can further promote hepatic IR [46]. Therefore, in humans, peripheral and hepatic IR are closely interrelated. Recent data on the role of the endocrine system and the hypothalamus in the development of and worsening of IR have been reviewed elsewhere [47].

B). The Role of IR in the Development of NAFLD and Cardiovascular Disease

Epidemiological studies suggest a strong relationship between IR and CVD in nondiabetic subjects [48]. IR is associated with dyslipidemia and other abnormalities, including oxidative stress, endothelial dysfunction and release of proinflammatory cytokines, that all contribute to the pathogenesis of CVD [49]

Similarly, IR plays a major role in the development and progression of NAFLD [50-53]. In the EGIR RISC Study, an ongoing prospective multicentre project involving a large European Caucasian population of non-diabetic subjects aiming to evaluate the relationship between IR and cardiovascular risk, fatty liver was associated with IR, higher Framingham risk scores and increased intima-media thickness suggesting an increased risk for CHD [54]. In turn, hepatic steatosis causes the release of pro-inflammatory cytokines such as Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) that contribute to fibrosis progression [55-57] and to premature arterial aging, hence the concept of an *"atherogenic liver"* [53].

The complex mechanisms that govern the development of steatosis and NASH, including host genetic variability, intestinal dysbiosis, adipose tissue dysfunction and intrahepatic molecular and cellular changes are beyond the scope of this review [58]. However, as IR is an independent risk factor for the development of NASH, it represents an ideal (but not the only) therapeutic target to prevent and treat NAFLD [50,59-61].

C). The Role of IR in the Development of HCC

For HCC occurring in the context of NAFLD/NASH, insulin and Insulin-like Growth Factor (IGF) type I receptor family (IIRF) are recognized to play a role in promoting tumor growth[62,63] . Most cancer types express both the insulin receptor and IGF genes, which represent a tyrosine-kinase class of membrane receptors that are homologous to oncogenes of the tyrosine-kinase class [64,65]. Similarly, there is a relationship between cancer and obesity, and cancer risk is closely associated with weight gain, hyperinsulinemia and IR [66-69]. Finally, IR leads to the release of multiple pro-inflammatory, oncogenic cytokines (such as Interleukin-6, tumor necrosis factor-α), while hyperinsulinemia stimulates the production of IGF-1 that promotes cellular proliferation and a reduction in apoptosis [70,71] .

D). Why Treat IR in NASH?

There are several cogent reasons for treating IR associated with NASH. First, IR is almost universal in NASH [50], which is associated with liver-related mortality and cardiovascular disease [1,72]. Second, patients with NASH are at a higher risk for developing HCC particularly if T2D coexists [73]. Whether and to what extent ISDs prevent HCC is a key question in the management of NAFLD. Third, IR contributes to the development and progression of both NASH and atherosclerosis in subjects with NASH. With regard to the latter, it is of interest that metformin reduces vascular stiffness in patients with NAFLD, further to, and independent of glycemic control [74].

E). Effects of Current Treatments of IR on NAFLD

Lifestyle changes that promote weight loss (diet and exercise) and bariatric surgery have proven effective in improving IR and reducing the extent of liver steatosis [75,76].

A 7-10% weight loss with intensive multidisciplinary lifestyle intervention is associated with improving hepatic steatosis, NAFLD activity score (NAS) but not fibrosis [77]. Likewise, exercise increases hepatic and extra-hepatic AMPK mediated non-esterified fatty acid oxidation, reduces post-prandial hepatic lipogenesis and proinflammatory cytokines release, reduces liver steatosis and improves ALT levels [78]. Physical exercise also improves glucose metabolism, insulin sensitivity, and atherogenic dyslipidemia without significantly affecting body weight [79]. In contrast, low-carbohydrate diets significantly reduces waist circumference further to improving pancreatic beta-cell function, triglyceride levels and insulin sensitivity [80] Weight loss >10% seems to be necessary to improve hepatic necroinflammation [81].

Bariatric surgery, indicated to treat severe obesity seems to reduce hepatic steatosis, steatohepatitis and fibrosis, and improves IR. However this form of therapy is not without complications and it is premature to consider foregut bariatric surgery as an established option to specifically treat NASH [82].

Recent analysis, however, is inconclusive as to whether the reduction in IR is sufficient to improve liver histology [83-85].

SECTION 2. METFORMIN IN PREVENTION AND TREATMENT OF NAFLD

A). Metabolic Effects of Metformin

Metformin is licensed for the treatment of type 2 diabetes mellitus at the recommended doses of 500-2500 mg daily. The mechanism of action of metformin, an oral biguanide glucose-lowering agent with effects on the mithocondria, has not been fully elucidated, but involves activation of the adenosine monophosphate-activated protein kinase (AMPK) pathway[86]. Activated AMPK, an evolutionarily conserved gauge of intracellular energy depletion, switches cell metabolism from an anabolic to a catabolic state, resulting in the inhibition of glucose, lipid and protein synthesis as well as cellular growth and increased fatty acid β-oxidation [87]

Glucose metabolism- Metformin decreases intestinal glucose absorption and increases insulin mediated glucose uptake in skeletal muscle by positive effects on insulin receptor, expression and tyrosine-kinase activity [88] . Moreover, metformin seems to increase plasma levels of GLP-1 and to induce islet incretin receptor gene expression through a PPAR-α mediated mechanism [89] . Metformin reduces hepatic gluconeogenesis linking the organic cation transporter 1 (OCT1) in hepatocytes, which facilitates the uptake of metformin [90] The final result is a reduction in hyperinsulinemia [91,9293].

Lipoprotein metabolism- AMPK activation by metformin induces the phosphorylation and inactivation of acetyl CoA carboxylase (ACC), an important rate-controlling enzyme for the synthesis of malonyl-CoA, which is both a critical precursor for the biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation [94] . Metformin participates in the regulation of lipogenic genes expression by down-regulating sterol regulatory element-binding protein-1c (SREBP-1c) gene expression [95]. As a result of these mechanisms, metformin improves lipoprotein profile

by decreasing LDL cholesterol levels and triglycerides, and increasing HDL cholesterol.

Others effects- Metformin, by decreasing levels of plasminogen activator inhibitor-1 and increasing tissue plasminogen activator activity, reduces markers of inflammation and levels of advanced glycated end products (AGEs), which are oxidative mediators of endothelial dysfunction [96]. Interestingly, metformin also exerts anti-hypertensive effects [97]. Moreover, compared to insulin, metformin use has recently been associated with a reduced risk of cancer, notably HCC [98-101] (Table **1**) [97,99,102-119], to which, as previously mentioned, diabetic patients appear to be particularly vulnerable [14]. Based on these properties, metformin is the "first choice" drug for the treatment of type 2 diabetes mellitus, MetS and polycystic ovary syndrome (PCOS), all conditions typically associated with NAFLD [106,120].

B). Metformin in NAFLD

Two meta-analyses of randomized-controlled trials (RCTs) have shown that metformin reduces both IR and MetS without improving hepatic histology in NASH [111,112] (Fig. **1**) [53].

Biochemical Response

In a 12-month prospective randomized, placebo-controlled trial comparing diet and exercise alone, to diet and exercise plus metformin in non-diabetic subjects with IR and NASH, metformin (maximum dose 1 gr daily) improved alanine aminotransferase (ALT) levels in all groups treated, but there was no correlation with histological changes [113,121,122]. In the Diabetes Prevention Program (DPP) trial, that compared the effects of a program of diet and exercise with treatment with either metformin or placebo in overweight or obese adults with elevated fasting glucose or impaired glucose tolerance, the improvement in ALT was more sustained if associated with weight reduction. This suggests that the effect of metformin on liver tests and hepatic steatosis is mediated primarily by weight loss [113,123].

Histological Response

Steatosis

Metformin is more effective than di*et al*one in reducing steatosis [124]. Metformin administered in association with antioxidants leads to a reduction of hepatic steatosis, without improving neither lobular inflammation or hepatocellular ballooning [121].

Elevated levels of Fetuin A, a liver-derived glycoprotein that critically affects key enzymes in lipid and glucose metabolism, impairs insulin signalling and positively correlates with steatosis, IR and MetS, [125]. Metformin reduces *in vitro* the hepatic expression of Fetuin A suggesting that the metabolic effects of metformin are mediated by Fetuin A [126,127].

Necroinflammation and Fibrosis

Compared to placebo, treatment with metformin leads to improved liver test and reduced IR without any significant differences in hepatic histology [128].

In one RCT involving nineteen consecutive non-diabetic, but insulin resistant subjects with biopsy proven NASH, no improvement in NAFLD activity score (NAS) [129] or fibrosis was observed, whereas steatosis significantly improved [128]. Similarly in another report of 20 NASH subjects, no improvement in ballooning or lobular inflammation was observed after a 12 month-course of metformin supplemented with the antioxidant methyl donor N-acetyl-cysteine [121].

Table 1: Biological effects of metformin in humans.

Biological Function Parameter/abnormality	Action of Metformin	Ref
Insulin-resistance	reduction	[102-104]
Intestinal glucose absorption	reduction	[105]
Glucose-lowering	allow	[106]
Skeletal Muscle gluconeogenesis	increase	[107]
Hepatic gluconeogenesis	reduction	[108]
Hyperlipidemia	reduction of total-C, LDL-C and TG levels	[109,110]
Development of the Metabolic Syndrome	reduction	[111]
Arterial hypertension	reduction	[97]
Improved liver histology in NAFLD	↕	[111,112]
Liver tests	improvement	[102,113]
Cardiovascular risk	reduction	[114-116]
Weight gain	reduction	[98,99]
Development of cancer	reduction	[80,100]

Legend: total-C= Total cholesterol; LDL-C= low-density lipoprotein; TG= triglycerides; ↕= null/inconsistent

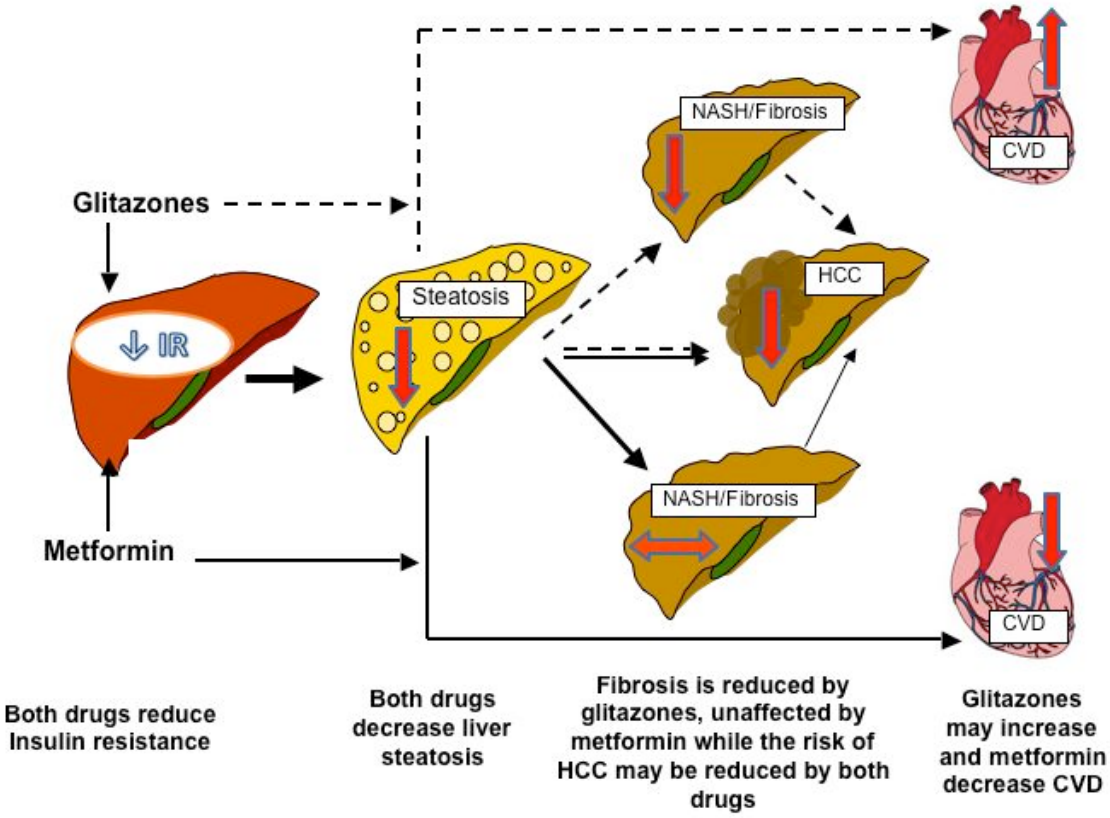

Fig. (1). Mechanisms of action and impact on natural history of NAFLD of glitazones and metformin [53]
Legend to (Fig. **1**): Metformin reduces IR via activation of AMPK, reduces liver steatosis, MetS and cardiovascular risk but does not improve liver histology. The PPARs-γ (glitazones) reduce IR via activation of multiple pathways, reduce liver steatosis and improve liver histology but are associated with worsening cardiovascular disease. IL-6= Interleukin-6; TNF-α= tumor necrosis factor- α; AMPK= 5'adenosine monophosphate-activated protein kinase; HCC= hepatocellular carcinoma; CVD= cardiovascular disease.

In their open-label RCT comparing the combination of rosiglitazone plus metformin to either drug alone in patients with NASH Omer and colleagues found that after 1 year of treatment, steatosis and necroinflammation significantly improved with rosiglitazone, but not with metformin. The combination of both drugs conferred no additional benefit to liver histology and glucose metabolism [130]. Thus, although it improves IS, metformin might fail to reverse the histological features of NASH. These findings contradict the results from an open label, randomized trial conducted in non diabetic patients, which reported that metformin at doses of 2 mg daily was more effective than either diet or Vitamin E in reducing steatosis, necroinflammation and fibrosis [113].

Finally, a recent meta-analysis of 78 RCTs confirmed that lifestyle interventions that induce weight loss or pioglitazone, but not metformin, improves liver histology and cardio-metabolic profile [131].

C). Metformin in Subjects with NASH and Increased CVD Risk

A subclinical proinflammatory state is the hallmark of cardiovascular risk in NAFLD. Compared to healthy controls, these patients typically display, significantly higher serum levels of proinflammatory cytokines such as C-reactive Protein (CRP), interleukin-6, and TNF-α. Furthermore, hs-CRP levels are higher in subjects with NASH than in individuals with simple steatosis [132,133]. Interestingly, Metformin reduces hs-CRP and contributes to reducing the pro-atherogenic stimulus in subject at higher CVD risk [122]. However, no published data specifically clarify whether metformin reduces CVD risk in subjects with NAFLD/NASH.

D). Metformin in HCC and Non-hepatic Cancer

Several observational and biological studies suggest a relationship between the use of metformin and a reduction in the incidence of various cancer types, including those of the breast, colon, ovary, lung and prostate [98,134-138]. Similarly, the use of metformin, compared to the use of sulphonylureas or insulin appears to be associated with a strong and statistically significant reduction in the risk of developing HCC, in diabetic patients [100,101]. The mechanisms for this effect are unclear. However, metformin appears to reduce carcinogenesis, in part, by inhibiting the synthesis of reactive oxygen species (ROS) production as a consequence of its effects on mitochondrial function [139], and by regulating the AMP-activated kinase (AMPK)/mammalian Target of Rapamycin (mTORC1) pathway favoring the anti-proliferative effects of AMPK [140,141]. Whether such a preventive effect is observed in non-diabetic NAFLD patients as well, however, remains to be ascertained.

E). Adverse Effects of Metformin

Having been used for over 30 years, metformin appears to be remarkably safe, with mild gastrointestinal effects alone in a minority of cases. However, its use is better avoided in case of impaired renal function, sepsis and congestive heart failure as in this context it is associated with a higher risk of lactic acidosis [84].

In conclusion, metformin alone does not appear to be effective for the treatment of NASH, but its use is appropriate in the subgroup of patients with MetS and increased cardiovascular risk. [93,1310,142] (Table **2a**) [102,121-123,128,130,143-145].

Table 2a: Effect of metformin on liver enzymes and histology in patients with Nonalcoholic Fatty Liver Disease

Author, year	Daily Dose	Liver Enzymes	Liver Histology	Design	Comment	Duration	Number patients	CVD outcomes
Marchesini G, 2001 [102]	1,5 g	improved	Not assessed	Open label, single arm	Liver volume Reduction	4 months	20	NA
Bugianesi E, 2005 [113]	2 g	improved	Reduction necroinflammation	Open label, RCT	Associated weight loss	12 months	55	NA
Uygun A, 2004 [143]	1,7 g	improved	No significative difference in necroinflammatory activity	Open label, RCT	Associated weight loss	6 months	18	NA
Nobili V, 2008 [144]	1,5	improved	No changes in fibrosis	Open label, RCT	In children with NAFLD	24 months	10	NA
de Oliveira CP, 2008 [121]	1 g	improved	No improvement in inflammation	Open label, single arm	Benefical effects in Metformin + NAC group	12 months	20	NA
Idilman R, 2008 [122]	1,7 g	improved	Improved steatosis; not significant improvement in fibrosis	Open label, RCT	Benefical effect on metabolic parameters and hs-CRP levels in Metformin group	12 months	74	NA
Haukeland JW, 2009 [145]	2,5-3	improved	No improvement	Open label, RCT	Reduction in serum lipid and glucose	6 months	48	NA
Shields VW, 2009 [128]	0,5-1	improved	No improvement	Open label, RCT	Benefical in metabolic effects	12 months	19	NA
Krakoff J, 2010 [123]	1,7	improved	No improvement	Open label, RCT	Benefical effect if associated to weight loss	3 years	2153	NA
Omer Z, 2010 [130]	1,7	No improvement	No improvement	Open label, RCT	Benefical effect in Metformin+Rosiglitazone group	6 months	64	NA

Legend: CVD= cardiovascular disease; hs-CRP= high-sensitivity C-reactive Protein; NA= not assessed; NAC= N-acetyl-cysteine; RCT= randomized clinical trial.

Its putative role in preventing HCC in those with type 2 diabetes mellitus adds to the attraction of this agent.

SECTION 3. THIAZOLIDINEDIONES IN THE PREVENTION AND TREATMENT OF NAFLD

A). Metabolic Effects of Thiazolidinediones

Thiazolidinediones (TZDs or glitazones: pioglitazone and rosiglitazone) are licensed for the treatment of type 2 diabetes mellitus.. Pioglitazone is administered at the recommended doses of 15-45 mg daily in a single dose. Rosiglitazone in administered at the recommended dose of 4-8 mg daily. TZDs act as agonists of the peroxisome-proliferator activated receptor-gamma (PPAR-γ), a transcription factor that regulates gene expression in liver, adipose, vascular endothelium, and muscle tissue. Physiologically, these agents promote the differentiation of pre-adipocytes into adipocytes and, anatomically, leads to a redistribution of triglycerides from liver and muscle (namely from an ectopic site) to adipose tissue (the physiological reservoir of fat). Clinically, this leads to improved IS, improved glycaemic control, and decreased hepatic steatosis which in art occurs via increased concentrations of the insulin sensitizing anti-steatotic adipokine adiponectin and consequent activation of the AMPK pathway, [146-150]. Recent reports suggest that the use of TZDs is associated with a reduced incidence of HCC in type 2 diabetes mellitus, perhaps via inhibition of promotion and progression of cancer growth [101,151].

There are unfortunately numerous unwanted side effects and potential risks associated with the use of TZDs, including weight gain, fluid retention, increased fracture rate, and possibly excess cardiovascular events [152,153]. (Fig. **1**) [53]. This latter is of particularly worrisome given that cardiovascular events are a major complication in the natural history of NAFLD [154]. A recent meta-analysis suggests an excess of bladder cancer in type 2 diabetes

Table 2b: Effect of glitazones on liver enzymes and histology in patients with Nonalcoholic Fatty Liver Disease

Author, year	Daily Dose	Liver Enzymes	Liver Histology	Design	Comment	Duration	Number patients	CVD outcomes
Sanyal AJ, 2004 [156]	Pioglitazone 30 mg + Vitamin E	improved	improved	RCT	Combination therapy was superior to Vitamin E alone in improving steatosis, necroinflammation and pericellular fibrosis	6 months	18	NA
Belfort, 2006 [157]	Pioglitazone 45 mg + hypocaloric diet	improved	Reduction steatosis and necroinflammation	RCT	The reduction of fibrosis do not differ from the placebo group.	6 months	45	No adverse effects
Ratziu V, 2008 [158]	Rosiglitazone 8 mg	improved	reduction steatosis but no improvement in necroinflammation and NAS score versus placebo	RCT	reduction in markers of insulin resistance versus placebo	12 months	63	No difference versus placebo
Aithal GP, 2008 [159]	Pioglitazone 30 mg + exercise and diet	improved	improvement in hepatocellular injury, Mallory- Denk bodies and fibrosis	RCT	increase in weight gain versus placebo	12 months	61	NA
Sanyal AJ, 2010 [160]	Pioglitazone 30 mg	improved	improvement in steatosis and inflammation, but no in fibrosis score	RCT	No hepatotoxicity	24 months	80	No difference versus placebo

Legend: CVD= cardiovascular disease; NA= not assessed; ; RCT= randomized clinical trial.

mellitus patients treated with TZDs, in particularly with pioglitazone, [155]but these data need to be confirmed.

B). TZDs in NAFLD

There has been significant interest in evaluating TZDs to treat NASH. Here we summarize and discuss the results of five RCTs and two open label trials using pioglitazone or rosiglitazone (Table **2b**) [156-160]

Metabolic Response

In nondiabetic patients, pioglitazone induces a significant improvement in IS as assessed by euglycemic clamps or surrogate markers (hyperinsulinemia or homeostasis model assessment [HOMA]) [160,161]. A key issue is whether the improvement in insulin sensitivity correlates with biochemical and/or histological responses. Two studies reported that improved hepatic insulin sensitivity was mirrored by aminotransferase reduction [157], and improved surrogate markers of systemic IR [157,158]. Interestingly, the latter improvement was correlated with a reduction in steatosis [158]. On closer examination however, metabolic and histological improvement do not always coincide [158,160]. For example, although virtually all patients (93%) experiencing a significant reduction in steatosis also had a reduction in HOMA score, this was also the case for a large proportion (59%) of patients with unchanged steatosis [158]. Moreover, a subsequent analysis found that even a strong reduction in HOMA levels does not predict an improvement in necroinflammation or fibrosis. This "dissociation" between the improvement of IR and unchanged liver histology has led our group to speculate that IR occurs very early in the pathogenesis of NAFLD and thus is a factor "necessary but not sufficient" for the development of liver disease [85].

Biochemical Response

Glitazones reduce aminotransferases levels [157,158]. ALT levels decline at week 4 and this effect is maintained during treatment. However, treatment longer than one year might not result in any further improvements [84,160].

Histological Response

Steatosis

Most trials show a reduction in steatosis compared to placebo, with a variable individual response ranging from 47% to 65% [157,158,160,162]. Only a single trial showed no difference between groups, perhaps because the patients had minimal steatosis (5-25%) at baseline, making differences between groups harder to discern [159].

Necroinflammation and Fibrosis

In published studies, assessment of inflammation is complicated by the differing histological scoring systems used. Ballooning improved in 32-54% of patients, significantly more than placebo in two RCTs [157,159]. Likewise, in the PIVENS study, the change in ballooning was higher with Pioglitazone than placebo [160]. Intralobular inflammation improved in all studies [157,159-161] but one [158]. Portal inflammation was unchanged or worsened with rosiglitazone [162].

Not all studies reported on changes in the NAFLD activity score (NAS). In a 6 month study, the score improved in 46% of patients treated with pioglitazone versus only 14% of the placebo

group (p=0.02). This could have been due to a reduction in steatosis which is part of the score. In contrast, a 1-year study with rosiglitazone failed to demonstrate any significant changes in the NAS score [157,,158], whereas a 2-year study with pioglitazone improved the NAS score significantly more than placebo [160] .

Even after prolonged treatment with rosiglitazone, fibrosis was not altered [84], including when assessed by micromorphometry, a more reliable quantitative technique [157,159,161].

C). TZDs and HCC and Non-hepatic Cancer

Peroxisome-proliferator-activated receptor gamma (PPARγ) plays a role in the development of some malignancies, including HCC [101,163]. *In vitro* and *in vivo* models indicate that the inhibition of PPARγ is able to inhibit HCC cell proliferation and tumor growth by inducing cell cycle arrest and apoptosis via the regulation of a panel of downstream effector molecules [164,165]. PPARγ inhibition also induces an inhibitory effect on HCC metastasis [166]. Therefore, PPARγ inhibition obtained through administration of TZDs could be anti-tumorigenic [167]. (Table **3**) [30,100, 101,165,168-171]

D). Adverse Effects of TZDs and their use in CVD

TZDs are nuclear receptor ligands and have several biological effects [172] including unwanted effects such as an increased risk of vascular events, precipitation or worsening of congestive heart failure, osteoporosis, weight gain, and bladder cancer. Increased cardiovascular risk appears to be drug-specific rather than a class effect. Further studies are required to evaluate the effectiveness and safety of glitazones for the treatment of NAFLD associated with either impaired glucose tolerance or overt type 2 diabetes mellitus. [174].

Rosiglitazone and Pioglitazone Vascular Disease

Atherosclerosis is one of the major complication of type 2 diabetes mellitus and NASH and the ideal ISD should protect these patients from excess cardiovascular risk[174,175]. Concerns remain as to whether rosiglitazone increases cardiovascular risks, but it is also clear that this drug does not substantially reduce these risks, a benefit that would be highly desirable during treatment of NAFLD alone or in associated with type 2 diabetes mellitus.

Unlike rosiglitazone, pioglitazone has not been associated with excess cardiovascular morbility and mortality, as demonstrated by the prospective, placebo-controlled PROactive trial [176,177].

Congestive Heart Failure

TZDs have been associated with an increased risk of congestive heart failure. The absolute risk, however, is small, amounting to 0.5% compared to 0.1% for placebo in the DREAM trial of rosiglitazone [178], 6% compared to 4% in the PROactive trial [176] of pioglitazone, and a doubling of relative risk upon meta-analysis [179]. Longer duration of use, age over 50, and obesity are predictive factors for developing congestive heart failure [177].

Bone Loss and Fractures

Schwartz *et al* showed that in patients with type 2 diabetes mellitus, TZDs use was associated with accelerated bone loss in women but not men [180]. Furthermore post-hoc analyses for fractures of industry-sponsored trial data confirms that rosiglitazone is associated with a doubling of the bone fracture rate in women, mostly in the arms, hands, and feet. Pioglitazone was similarly associated with increased fractures in the distal arm and legs. The mechanism is thought to relate to interference of bone formation and turnover by TZDs.

Weight Gain

TZDs induce in some but not all patients, a weight gain of ~4 kg [158,162,176]. This effect is due to expanded peripheral rather than visceral adipose tissue and thus does not increase the metabolic and inflammatory abnormalities associated with intra-abdominal adiposity [181] but rather mirrors redistribution of fat to "where it belongs" and is compounded by concurrent fluid retention [182,183].

In conclusion, TZDs modestly improve histological parameters in NAFLD, but induce weight gain and so further studies are needed to demonstrate the long term beneficial effects of these drugs for routine use in the prevention and therapy of NAFLD [184]. TZDs prevent the development type 2 diabetes mellitus and seem to be suitable for use in the subgroup of subjects with impaired glucose tolerance and no heart failure [178,185].

SECTION 4. NAFLD AND INSULIN SENSITIZERS IN CHILDHOOD

As a result of the overweight and obesity epidemic, NAFLD is the most common cause of chronic liver disease and a leading cause of liver transplantation in children and teen agers in the United States [186,187]. NAFLD in children has a different histological spectrum, but as in adults, can evolve to cirrhosis and is associated with increased cardiovascular risk [188-173].

As in adults, therapeutic approaches in children are directed to reduce IR but, to date, there are no proven therapies that halt progression or improve prognosis [58,193]. ISDs such as metformin and TZDs have been used, although their long-term efficacy and safety remain unknown [194]. The TONIC trial was a double blind, multicenter randomized trial evaluating NAFLD treatment using histologic outcomes. It involved 173 children aged 8 to 17 years randomized to receive either metformin, Vitamin E or placebo [195]. In the subgroup treated with metformin, improvements in ALT and hepatocellular ballooning were detected compared to the placebo group, although no significant changes were demonstrated in steatosis, inflammation or the NAS score over a 96 week follow-up. Lifestyle changes aiming to reduce obesity have proven effective in all age groups and therefore should be adopted [196,197].

SECTION 5. INSULIN SENSITIZERS BEYOND METFORMIN AND THIAZOLIDINEDIONES: WHAT'S NEXT?

Other ISDs that are potentially useful in human NASH are being developed or tested.

Recent studies have shown a direct and beneficial effect on hepatocytes of **glucagon-like peptide-1 (GLP-1)**, a class of drugs used for the treatment of type 2 diabetes mellitus. GLP-1, acts by activating genes involved in β-oxidation of fatty acids and insulin-sensitization [198,199]. In terms of clinical use exenatide, a synthetic version of exendin-4, a hormone initially isolated from the saliva of the Gila monster was approved by the FDA for use in the US in 2005 as an adjunctive therapy for type 2 diabetes mellitus and is available as a subcutaneous injection. This peptide is a GLP-1 receptor agonist and primarily stimulates the release of insulin from pancreatic β-cells. However, exenatide does not act as a direct insulin sensitizer, but rather induces clinically significant weight loss, which may eventually lead to an insulin-sensitizing effect. In ob/ob mice, exendin-4 significantly reduced blood glucose, improved IS, and reduced hepatic steatosis[200]. GLP-1 proteins have a novel direct effect on hepatocyte fat metabolism [201].

An open-label, uncontrolled clinical trial using exenatide to assess drug safety in diabetics over a period of ~3.5 years revealed that patients had improved AST and insulin sensitivity [202]. In addition, those with elevated ALT at baseline (n=116) had significant reductions in ALT, while 41% achieved normal levels on treatment. Patients with elevated ALT compared to those with normal ALT levels at baseline tended to lose more weight. However, weight change was not correlated with baseline ALT values nor were changes in ALT. There is one case report of a 59-year-old male with type 2 diabetes mellitus who was treated with exenatide and metformin [203]. Following a 44- week course of exenatide, this patient displayed normalized ALT, and spectroscopic evidence of decreased hepatic steatosis (from 15.8% to 4.3%).

Table 3. Insulin sensitizers and prevention of hepatocellular carcinoma

Author	Population studied	Drugs	Methodology	Results	Conclusion
GLITAZONES					
Chang CH 2012 [165]	606,583 diabetic patients from Taiwan followed prospectively from 2000 to 2007	Rosiglitazone and Pioglitazone	Prospective case-control study	Incidence HCC rosiglitazone: OR= 0.73 (95% CI: 0.65-0.81); pioglitazone: OR= 0,83 (95% CI: 0,72-0,95)	Decreased liver cancer incidence with pioglitazone for higher cumulative dose and longer duration.
Lai SW, 2012 [101]	Taiwan National Health Insurance Research Database, 19,349 newly diagnosed T2D patients and 77,396 comparison subjects without DM were identified.	Metformin and thiazolidinediones	Population-based observational study from 2000 to 2005.	Occurrence of HCC: HR at 0,49 (95% CI= 0,37-0,66) in subjects taking metformin; at 0,56 (95% CI= 0,37-0,84) in subjects taking thiazolidinediones	The use of metformin or thiazolidinediones may reduce the risk of developing HCC (51% versus 44% reduction, respectively) in high risk patients.
Hassan MM, 2010 [168]	140 diabetic patients with HCC	Insulin, metformin, sulphonylureas, thiazolidinediones	Hospital-based case control study	The adjusted OR for HCC association was 0,3 (CI= 0,1-*0,7;* P= 0,01) in thiazolidinediones users, similarly with metformin users (adjusted OR=0,3, CI 0,2-0,6; P= 0,001)	
METFORMIN					
Donadon V, 2010 [100]	610 HCC patients compared with 618 matched cirrhotic patients and 1696 Controls.	insulin, sulphonylureas and metformin	Retrospective, hospital-based, case-control study	metformin compared with sulphonylureas: OR for HCC of 0.15 (CI= 0.04–0.50; *P*=0.005); metformin compared with insulin: OR=0.16 (CI 0.06–0.46; *P*=0.0006)	In diabetic patients, treatment with metformin was associated with a strong and statistically significant reduction of the risk of HCC, if compared with the use of other drugs.
Chen TM, 2011 [169]	53 diabetic patients with early-stage HCC undergoing RFA	Metformin versus other treatment (sulphonylureas, insulin)	Retrospective analysis of a cohort of a single-hospital database.	Diabetic patients treated with metformin had better survival outcome compared to patients without metformin treatment: adjusted HR 0,24 (95% CI: 0,07-0,80, *P*=0,020)	No difference in mortality rate between patients taking metformin and nondiabetic patients. Sulphonylureas and insulin treatment did not achieve significant conclusions.

(Table 3) Contd….

Author	Population studied	Drugs	Methodology	Results	Conclusion
Nkontchou G, 2011 [170]	100 consecutive diabetic patients with HCV cirrhosis included in a screening program for HCC.	Metformin	Observational prospective cohort (1988-2007) at a university hospital referral center.	Occurrence of HCC: HR at 0,19 (95% CI= 0,04-0,79, *P*= 0,049) in group treated with metformin.	Treatment with metformin reduces incidence of HCC and liver-related mortality in patients with diabetes and HCV.
Lai SW, 2012 [101]	Taiwan National Health Insurance Research Database, 19,349 newly diagnosed T2D patients and 77,396 comparison subjects without DM were identified.	Metformin and thiazolidinediones	Population-based observational study from 2000 to 2005..	Occurrence of HCC: HR at 0,49 (95% CI= 0,37-0,66) in subjects taking metformin; at 0,56 (95% CI= 0,37-0,84) in subjects taking thiazolidinediones	The use of metformin or thiazolidinediones may reduce the risk of developing HCC (51% versus 44% reduction, respectively) in high risk patients.
Chen HP, 2012 [171]	97430 HCC patients, 194860 controls	metformin	Population-based case-control study	In diabetics, adjusted HCC OR= 0,93 (95% CI 0,91-0,94, p<0,0001)	In diabetic patients, each incremental year increase in metformin use resulted in 7% reduction in the risk of HCC.
Wang P, 2012 [30]	More than 2 000 000 partecipant with diabetes were included in meta-analysis	metformin	Meta analysis of 17 case-control studies and 32 cohort studies	Pooled risk of HCC estimates were 0,31 (95% CI= 0,19-0,49) for patients receiving metformin, and 4,0% (95% CI= 1,94-8,24) for patients receiving sulphonylureas or insulin treatment.	Metformin reduces incidence of HCC in diabetic patients, in comparison to other antidiabetic drugs.

The long-acting glucagon-like peptide **liraglutide** also improves insulin sensitivity and reduces lipid accumulation in liver through multiple and incompletely understood mechanisms [204, 205].

Liraglutide, has 97% amino acid sequence identity to native human GLP-1 and an acyl side-chain attachment, which makes it bind to albumin. These small structural differences prolong the half-life of GLP-1 to 13 hours, making it possible to administer daily. Several studies showed that liraglutide was well tolerated, improved glycaemic control with a low risk of hypoglycemia, improved beta-cell function, and was associated with weight reduction. The receptors of GLP-1 analogues also exist in human hepatocytes and administration of GLP-1 analogues are reported to directly reduce liver steatosis and fibrosis *in vivo* [201,206]. More studies are underway to ascertain its effect in NAFLD/NASH[207].

Similarly, the dipeptidyl peptidase-IV inhibitor **(DPP-IV)** sitagliptin improves postprandial insulin secretion, reduces excess glucagon secretion, promote saxiety, ameliorates liver tests in T2D and affects liver fibrogenesis either via increasing GLP-1 activity and/or anti-inflammatory activities in liver [208-211]. In a recent open-label, single-arm observational pilot study, treatment with sitagliptin (100 mg once a day per 12 months) ameliorated liver enzymes and hepatocyte ballooning in NASH patients with type 2 diabetes mellitus [212].

Other PPAR agonists have been shown to have insulin-sensitizing effects and thus are potentially indicated for use in NAFLD. These include the **PPAR-δ agonist** GW501516 which has been examined in a mouse model of NASH [213]. GW501516 reduced hepatic triglyceride, hepatic fat droplets, inflammatory cells, and decreased the expression of pro-inflammatory markers. Likewise, PPAR-δ agonist treatment in an ethanol-mediated hepatic injury and steatosis rat model attenuated the severity of adverse effects from ethanol on hepatic repair by restoring insulin responsiveness [214]. Collectively, these findings suggest that PPAR-δ is a potential therapeutic target for IR and hepatic steatosis [215,216].

Finally, there was initial promise with the **cannabinoid type I (CB1) receptor blockers** in improving hepatic steatosis and promoting weight loss in NAFLD. The endocannabinoid system is involved in the regulation of food uptake, body weight, and insulin sensitivity. Obesity leads to up-regulation of the CB1 receptors, which leads to hepatic lipogenesis, fatty acid synthesis in adipocytes, and decreased adiponectin levels [217]. In two large, placebo-controlled clinical trials using the CB1 antagonist **rimonabant** to study weight loss, metabolic improvements were remarkable with notable improvements in insulin sensitivity [218,219]. This was largely thought to be due to weight loss, but the metabolic effects appear to exceed what is directly related to weight loss alone, suggesting a direct action in improving insulin sensitivity. While there were several CB1 antagonists in development (Rimonabant (SR141716), taranabant, and otenabant), enthusiasm for these agents has waned after Rimonabant, was withdrawn from the market in the European Union due to adverse psychiatric effects, mostly severe depression, and suicidal behavior [220]. Current research is devoted to developing peripherally selective CBI antagonists devoid of the psychiatric adverse effects.

Table 4. Comparison of the Effectiveness of the Various Insulin Sensitizers in NASH

Drug	NAFLD			Complications of NAFLD				REFERENCES
	Liver enzymes	Liver steatosis	Liver fibrosis	HCC	non-HCC Cancer	CVD	MetS	
Metformin	reduction	reduction	indifferent	reduction	reduction	reduction	reduction	[62,102,102,112,115,116,134,135,137,262]
Glitazones	reduction	reduction	reduction	reduction	reduction	worsening	reduction	[101,115,263]
DPP-IV inhibitors	reduction	reduction	?	?	?	reduction	reduction	[210,211,264,265]
GLP-1 agonists	reduction	reduction	reduction/?	?	?	reduction	reduction	[198-200,202,204,266-267]
Lipid-lowering drugs	reduction	reduction	?	reduction	reduction	reduction	reduction	[227,270-274]
Antioxidants	reduction	reduction	?	?	?	worsening or no effect	?	[160,231,232,275-277]
UDCA	reduction	reduction	?	?/ worsening	?/ worsening	benefical	benefical	[221-223,278,279]
Angiotensin receptor blockers (AT-1)	reduction	reduction	reduction	?/ reduction	?/ reduction	reduction	reduction	[280-283]

Legend: NAFLD= nonalcoholic fatty liver disease; HCC= hepatocellular carcinoma; CVD= cardiovascular disease; MetS= Metabolic Syndrome; DPP-IV= dipeptidyl peptidase IV; GLP-1= glucagon-like peptide 1; UDCA= Ursodeoxycholic acid; Antioxydant= Vitamin E, betaine, N-acetyl-cysteine; Lipid-lowering drugs= statins, fibrates, niacin, ezetimibe, n-3 polyunsaturated fatty acids (PUFAs).
"?"= undetermined effect.

Impaired regulation of bile acid metabolism may contribute to the development of NAFLD. Ursodeoxycholic acid **(UDCA)** improves hepatic metabolism and insulin sensitivity by multiple effects including the regulation of *de novo* lipogenesis, stimulation of glucagon-like peptide-1 secretion in the small intestine, and improved energy homeostasis in brown adipose issue and skeletal muscle [221,222]. However clinical trials of these agents in NASH have provided negative results and bile acids are not recommended [223].

Statins have immunomodulatory and anti-inflammatory actions, useful in the treatment of both CVD and NASH [224-229]. Individual statins such as rosuvastatin have been shown to exert a beneficial effect in improving insulin sensitivity in animal models of NAFLD [230]. However there are no sufficiently powered studies to demonstrate a beneficial effect of these agents on liver histology in human NAFLD/NASH.

Oxidative stress induced by lipid peroxidation and the production of reactive oxygen species (ROS) are key-mechanisms for the development of NASH and cardiovascular disease. **Antioxidant agents** (including Vitamin E, betaine and N-acetyl-cysteine) may therefore be effective in treating NASH and improve liver enzymes and histology as recently demonstrated by the PIVENS trial [160]. However, the data need to be replicated in well-designed randomized controlled trials that are adequately powered and of a sufficient duration to determine clear efficacy [160,231,232].

Adiponectin is an adipocytokine with pleiotropic effects acting principally through AMPK pathway activation [233-235] including a reduction in the formation of vascular plaques, improving insulin sensitivity and glucose control, and anti-inflammatory and anti-fibrogenic actions in the liver which results in protective effects against HCC [236-240].

Adiponectin is a hepatic insulin sensitizer and inhibitor of tumor necrosis factor, and in NAFLD, low adiponectin levels are associated with reduced steatosis and inflammation [241-243]. Conversely, elevated serum concentrations of adiponectin are associated with protective effects against hepatic steatosis [60, 244, 245]. Thus, adiponectin agonists may be beneficial for the treatment of NAFLD in future.

Estrogens - Interestingly, estrogens may be considered as ISDs [246-248]. Estrogen receptors (ER) are expressed in many tissues including the liver and white adipose tissue, and estrogens act via nuclear and extranuclear pathways [249,250]. Estrogens stimulates leptin synthesis and secretion via ER-dependent transcriptional mechanisms, leading to metabolic regulation [251]. The finding that human NAFLD demonstrates sexual dimorphism in its manifestations [252-254], that treatment with the antiestrogen tamoxifen [255] and naturally occurring hyperandrogenism in PCOS [256] are associated with NASH and that hormonal replacement therapy is associated with antifibrotic activity in women with chronic hepatitis C [257] and that estrogens exert hepatoprotective effects *in vitro*

[258], all suggest that estrogens may have a role in the management of NAFLD. However, no clinical data are available in this regard.

CONCLUSIONS

Suggestions for the use of the most relevant ISDs in clinical practice have been detailed in conclusions to Section 2 and Section 3. Certain agents such as metformin and thiazolidinediones might be appropriate for early stage disease (i.e. pure steatosis). In cases of NASH/cirrhosis, particularly in those with decompensated liver disease, metformin should be used with caution. The strong pathogenic association of IR with NAFLD justifies further consideration of the use of ISDs. Available data suggest that the pathogenesis of liver injury in this disease is multi factorial [259] and clinical trials of a combined therapeutic approach including various ISDs classes needs to be considered. [260,261].

Finally, fully innovative research avenues are disclosed by the finding that other novel "non-classic" insulin sensitizers may be of potential utility in the prevention and treatment of NAFLD and its metabolic, hepatic and extrahepatic vascular and oncologic complications (Table **4**). [62,101,102,111,115,116,134,135,137,160,198-200,202,204,210,211,221-223,227,231,232,262-283]

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

ALT	=	Alanine aminotransferase
CHD	=	Coronary heart disease
CVD	=	Cardiovascular disease
DPP-IV	=	Dipeptidyl peptidase-IV inhibitors
GLP-1	=	Glucagon-like peptide-1
HCC	=	Hepatocellular carcinoma
IR	=	Insulin Resistance
ISDs	=	Insulin-sensitizer drugs
MetS	=	Metabolic Syndrome
NAFLD	=	Nonalcoholic fatty liver disease
NAS	=	NAFLD score
NASH	=	Nonalcoholic steatohepatitis
PCOS	=	Polycystic ovary syndrome
T2D	=	Type 2 diabetes mellitus
TZDs	=	Thiazolidinediones
TGs	=	Triglycerides
UDCA	=	Ursodeoxycholic acid

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