Modulation of sterol regulatory element binding proteins (SREBPs) as potential treatments for non-alcoholic fatty liver disease (NAFLD)

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Non-alcoholic fatty liver disease (NAFLD) is associated with diabetes, obesity and insulin resistance. The pathogenesis of NAFLD is complex, but modulation of the activities of transcription factors that regulate hepatic lipid and glucose homeostasis may be a key to treating NAFLD. An example of a key transcription factor regulating hepatic lipid metabolism is sterol regulatory element binding proteins (SREBPs), and in this review we present evidence supporting a key role for SREBPs in NAFLD. Currently, the only effective treatment for NAFLD is caloric restriction and peroxisome proliferator activated receptor (PPAR-γ) agonists. We suggest that further studies are urgently needed to evaluate modulation of SREBP activity as a potential new treatment for NAFLD.

Introduction

Fatty liver is a common histological finding in human liver biopsy specimens. Fatty liver affects 10–24% of the general population and may be a marker of risk for future chronic liver disease. Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver dysfunction and it has been estimated that, with an increasing prevalence of obesity, 20 million patients are affected in the USA. NAFLD represents a spectrum of diseases ranging from simple fatty liver (steatosis) to steatosis with inflammation and necrosis to cirrhosis. Non-alcoholic steatohepatitis (NASH) represents the more severe end of this spectrum and is associated with progressive liver disease, fibrosis and cirrhosis [1] The major risk factors for NAFLD are obesity and insulin resistance, and the prevalence of these risk factors has increased rapidly worldwide [1]. NAFLD is rapidly becoming an important problem for patients and health care providers. Undiagnosed, this condition may progress silently and result in cirrhosis, portal hypertension and liver-related death in early adulthood [2]. A recent study by Adams *et al.* [3] of 420 patients diagnosed with NAFLD, who were followed up between 1980 and 2000, showed that survival was lower in patients with NAFLD than the expected survival for the general population. The prevalence of diabetes was 22%, dyslipidaemia 23%, hypertension 22% and cirrhosis 5%. Overall, fibrosis, cirrhosis and hepatocelluar carcinoma developed in a small proportion of patients over 10 years, but the challenge for the future is to predict accurately those patients who will develop worsening liver disease over time.

NAFLD is now regarded as a hepatic component of the metabolic syndrome [1]. Mass screening for significant liver injury in patients with NAFLD will be an important medical challenge in the years to come because of the epidemics of obesity and diabetes. The inability of liver biopsy to provide simple diagnostic test makes the development of non-invasive diagnostic markers a high priority. However, currently there is no specific and sensitive single biochemical marker for NAFLD [4].

Recently, the diagnosis of NAFLD was suggested to be associated not only with increase in the incidence of diabetes but also with cardiovascular disease [5]. Inevitably this will increase the economic burden of NAFLD, and hence the need for pharmacological treatments is fully justified.

To date, the only effective treatment of NAFLD is caloric restriction, which is difficult to achieve for most NAFLD patients [1]. The current evidence suggests that treatments showing most promise are the peroxisome proliferator activated receptor (PPAR-γ) agonists acting to decrease hepatic lipid accumulation and attenuate the inflammatory response [6,7]. Peroxisome proliferator activated

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receptors (PPARs) are members of the nuclear receptor superfamily that may be involved in the pathogenesis of NAFLD and are key regulators of adipogenesis [6,7].

In this review we focus on the potential modulation of activity of the sterol regulatory element binding proteins (SREBPs) as potential targets for pharmacological interventions aimed at reducing fat accumulation and insulin resistance.

Sterol regulatory element binding proteins (SREBPs)

Sterol regulatory element binding proteins (SREBPs) are important transcription factors that regulate hepatocyte cholesterol homeostasis. SREBPs activate the expression of more than 30 genes regulating the synthesis and uptake of cholesterol, fatty acids, triglycerides and phospholipids, as well as the NADPH cofactor required to synthesize these molecules [8,9]. In the liver three SREBPs regulate the production of lipids for export as lipoproteins and as bile. The mammalian genome encodes three SREBP isoforms, designated SREBP-1a, SREBP-1c and SREBP-2. The promoter of the SREBP-1c gene contains response elements for insulin, glucagon and liver X-activated receptors (LXR). Knockout mice that lack all SREBPs die at an early stage of embryonic development. Specific knock out of SREBP-2 also results in embryonic lethality. Deletion of SREBP-1a allows some foetuses to survive, whereas lack of SREBP-1c appears to be of little consequence [10].

Overexpression of SREBP-1c in the liver of transgenic mice produces a triglyceride-enriched fatty liver with no increase in cholesterol, while SREBP-2 overexpression in transgenic mice resulted in a 28-fold increase in cholesterol synthesis [11]. In rat hepatocytes, insulin treatment increases the amount of SREBP-1c. The total amount of SREBP-1c in liver and adipose tissue is reduced

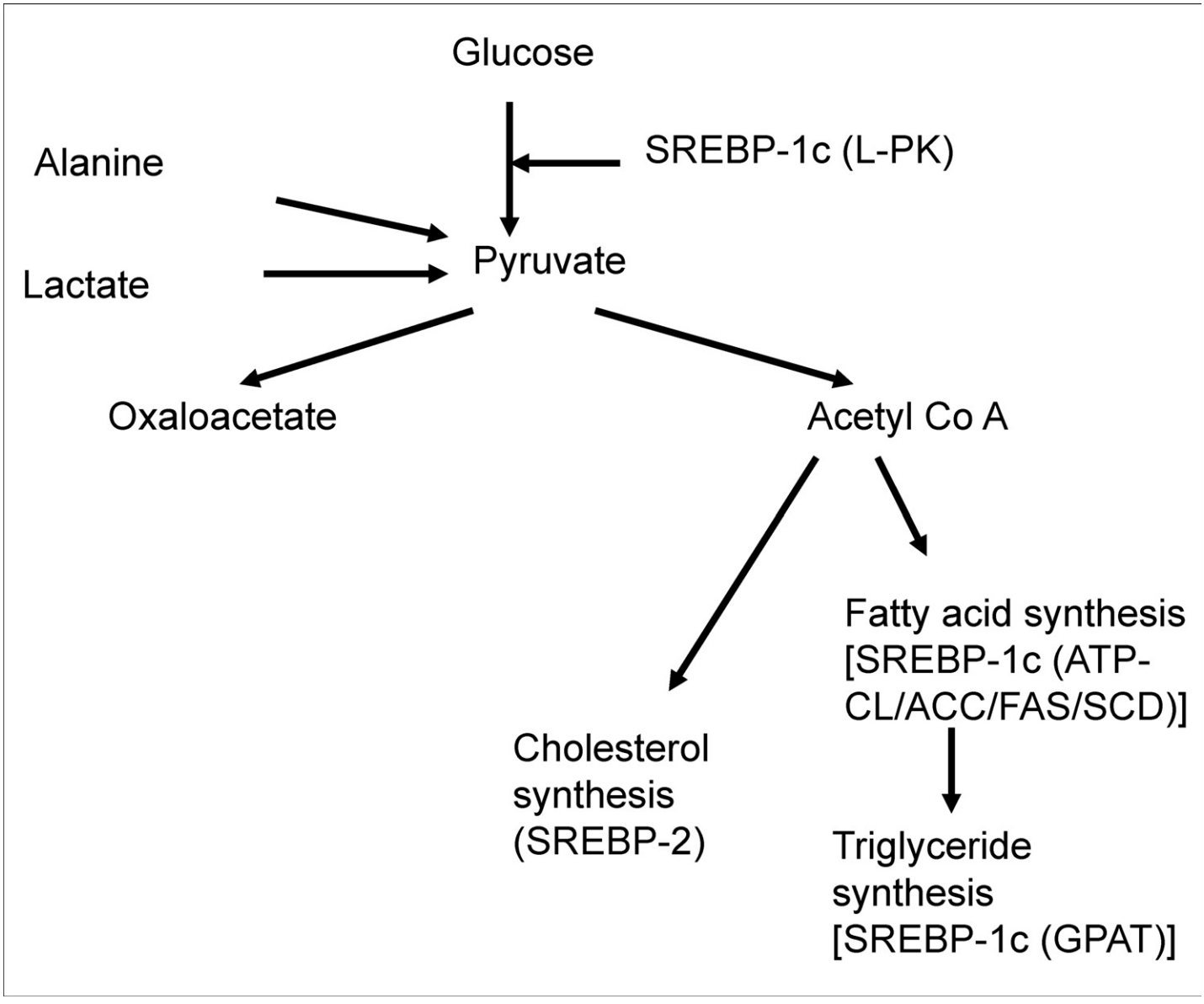

FIGURE 1

Key pathways in hepatic carbohydrate, fatty acid, triglyceride and cholesterol metabolism that are regulated by SREBPs and are relevant to hepatic lipid synthesis and NAFLD. Abbreviations used for key relevant enzymes are GK, glucokinase; L-PK, liver pyruvate kinase; ATP-CL, ATP citrate-lyase; ACC, acetyl CoA carboxylase; FAS, fatty acid synthase; SCD, steroyl CoA desaturase; GPAT, glycerol phosphate acyl transferase. L-PK, ACC, FAS and SCD require high carbohydrate intake and insulin concentrations to be induced by SREBP 1c. GK requires insulin to be induced by SREBP1c, whereas ATP-CL and GPAT are induced by SREBP1c alone.

by fasting, which suppresses insulin and increases glucagon levels, and is elevated by re-feeding [11,12]. SREBP-1c levels decrease when rats are treated with streptozotocin, which abolishes insulin secretion, and rise after insulin injection. In livers of knockout mice that lack all SREBPs in the liver there is a marked decrease in the insulin-induced stimulation of lipogenic gene expression [11]. Furthermore, SREBP-1c may also contribute to the regulation of glucose uptake and glucose synthesis through induction of expression of glucokinase, a key enzyme in glucose utilization. Fatty liver with insulin resistance is caused by SREBP-1c, which is increased in response to the high-insulin levels. Thus, SREBP-1c may play a crucial role in the regulation of hepatic glucose production and triglyceride. We suggest that because SREBP 1c represents a key modulator of hepatic glucose and triglyceride metabolism, SREBP activity may be a key factor involved in lipid accumulation in NAFLD.

Figure 1 shows the relevant pathways in hepatic carbohydrate and lipid metabolism regulated by SREBPs that may be involved in NAFLD.

Figure 2 is a schematic figure illustrating regulation of SREBP-1c by cholesterol metabolites and by therapeutic agents.

SREBPs and insulin resistance

The liver plays a major role in the regulation of glucose, lipid and energy metabolism. Hepatic fat accumulation is commonly associated with resistance to insulin's action to suppress hepatic gluconeogenesis and hepatic glucose output on hepatic glucose metabolism and antilipolysis in adipocytes. Interestingly, insulin resistance *per se* is associated with hepatic fat accumulation, independently of BMI and intra-abdominal obesity, and in individuals with NAFLD there is failure of insulin to suppress adequately plasma NEFA concentrations [13,14]. In the liver, insulin regulates fasting glucose concentration by inhibiting hepatic glucose production and stimulating glycogen synthesis. Hepatic glucose production involves balancing two metabolic pathways: glycogenolysis and gluconeogenesis. Once glycogen stores are replenished, glucose enters the glycolytic pathway and thereby provides carbon for *de novo* lipogenesis. Lipids are then stored as triglyceride or exported from the liver as VLDL [1,11]. Consistent with its function as anabolic hormone, insulin promotes the synthesis of hepatic triglyceride. High glucose and insulin concentrations inhibit fatty acid oxidation and when glucose is delivered to the liver in large quantities, glucose is converted to glycogen and stored. Insulin-regulated lipid and glucose homeostasis is modulated by SREBPs [15,16]. Insulin and SREBP-1c stimulate key lipogenic genes, including those encoding acetyl-CoA carboxylase and fatty acid synthase [9,17]. In such conditions both reduced fatty acid oxidation and increased *de novo* synthesis of fatty acids contribute to accumulation of fat in the liver in insulin-resistant individuals. Importantly, SREBP-1c not only competitively inhibits the action of PPAR-γ but also induces insulin resistance by inhibiting hepatic IRS2 signalling [18]. These effects

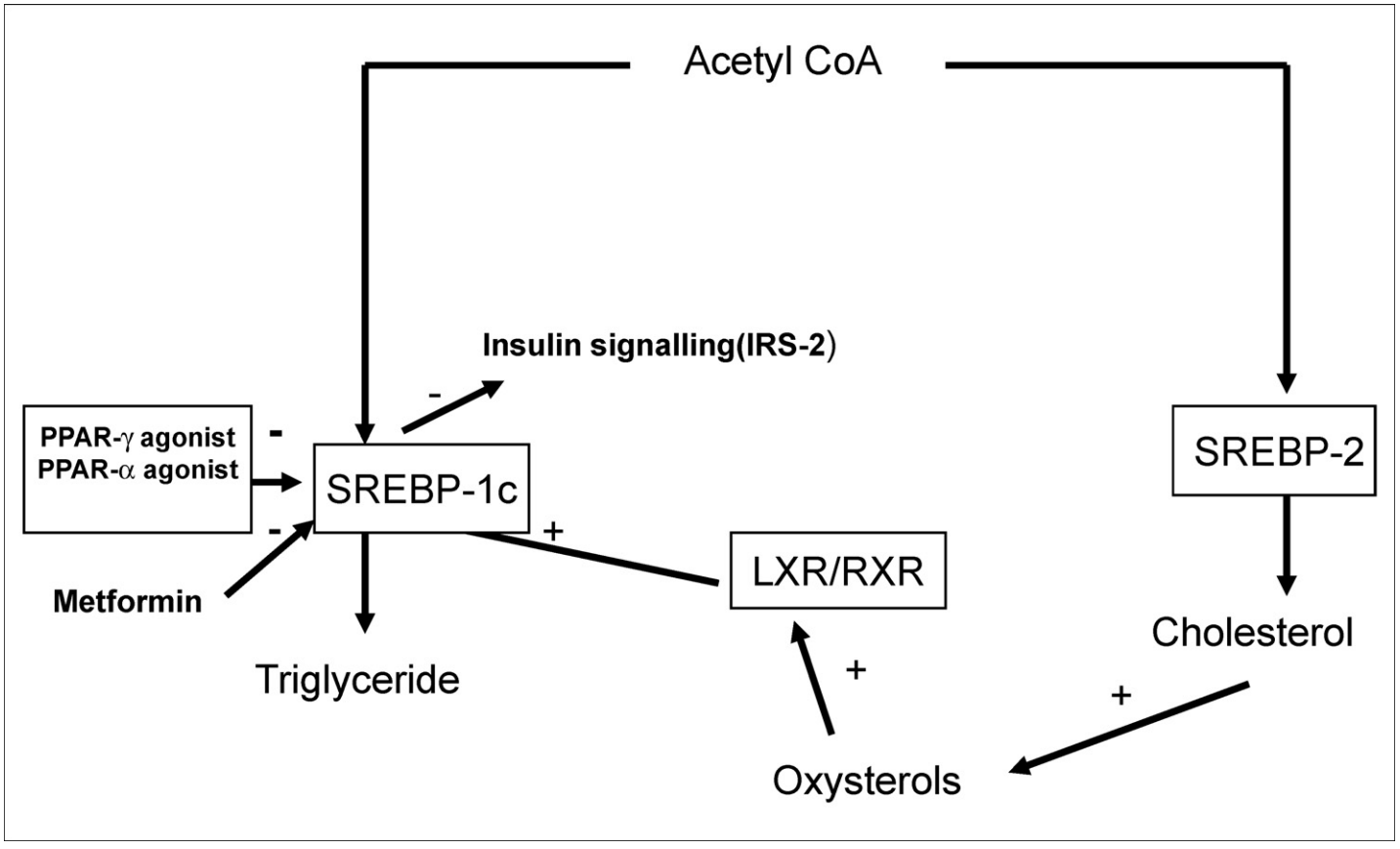

FIGURE 2

Schematic figure showing potential key metabolic pathways regulated by SREBPs relevant to NAFLD. The figure illustrates how hepatic triglyceride content may be modified by cholesterol metabolism, and by metformin, PPAR α and PPARγ agonists. The transcription factors involved in regulation of lipid biosynthesis are shown enclosed in boxes.

will lead to an increase in hepatic gluconeogenesis and stimulation of SREBP-1c, which leads ultimately to more accumulation of triglyceride in the liver. Consequently, pharmacological agents that modulate the action of SREBP-1c may have potential for treating NAFLD.

Furthermore, SREBP-1c levels are increased in the fatty livers of obese (*ob*/*ob*) mice with insulin resistance and hyperinsulinaemia caused by leptin deficiency. The increase in SREBP-1c increases lipogenic gene expression, enhances fatty acid synthesis and accelerates triglyceride accumulation [8,9]. Administration of leptin, which opposes SREBP-1c action, reversed these metabolic abnormalities [19]. Metformin reduces hepatic SREBP-1c levels and lowers lipid accumulation in livers of insulin-resistant *ob*/*ob* mice [17]. Metformin's glucose-lowering effect results from decreased hepatic glucose production and increased glucose utilization. Metformin's beneficial effects on circulating lipids have been linked to a potential benefit of metformin to decrease liver fat. Metformin indirectly activates AMP-activated protein kinase (AMPK) by activating the tumour-suppressor gene LKB1. AMPK is a major cellular regulator of lipid and glucose metabolism. By activating AMPK in hepatocytes, meformin should decrease *in vivo*, acetyl-CoA carboxylase (ACC) activity and thereby increase fatty acid oxidation and theoretically decrease hepatic fat accumulation. Activation of AMPK by metformin also suppresses expression of SREBP-1. Interestingly, in isolated rat skeletal muscles, metformin stimulates glucose uptake coincident with AMPK activation. Theoretically activation of AMPK provides an explanation for many of the pleiotropic beneficial effects of this drug [12]. Interestingly, TZDs not only activate AMPK through a signal pathway that is different from metformin but also increase plasma adiponectin that will potentially also further activate AMPK. The increase in plasma adiponectin concentration is associated with a decrease in accumulation of fat in the liver and improvement in insulin resistance. Furthermore, adiponectin also activates AMPK and inhibits ACC in skeletal muscle and fat. It is tempting to speculate, therefore, that metformin, TZDs, adiponectin and calorie restriction activate AMPK, and in turn modulate the action of SREBP-1 [20,21].

It would appear that fatty liver frequently observed in insulin-resistance maybe a result of high SREBP-1c levels caused by high-insulin levels. In livers of knockout mice that do not express SREBPs there is a marked decrease in the insulin-induced stimulation of lipogenic gene expression [11].

Fasting has been shown to reduce the total amount of SREBP-1c in the liver and adipose tissue [8]. Therefore, nutritional regulation of SREBP-1c and lipogenic gene expression may play a crucial role in the pathogenesis of NAFLD that is independent of insulin. For example, it has been suggested that polyunsaturated acids suppress the activation of SREBP-1c and ultimately contribute to reducing hepatic fat accumulation [22].

Furthermore, it has been suggested that SREBP-1c acts in synergy with the carbohydrate response element-binding protein (ChREBP) to induce glycolytic and lipogenic gene expression. ChREBP is a transcription factor that regulates carbohydrate (glucose)-mediated stimulation of lipogenesis. Glucose activates

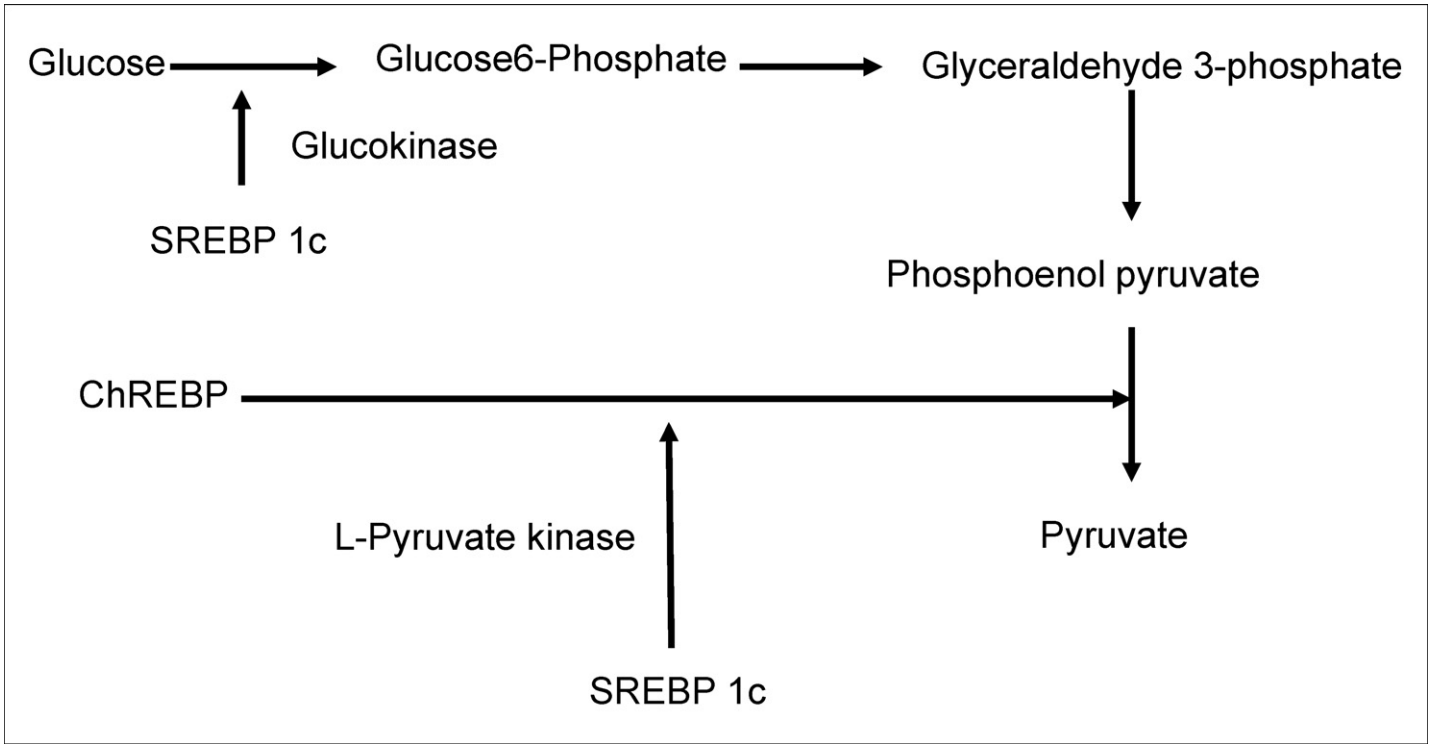

FIGURE 3

The role of ChREBP and SREBP1c in the hepatic metabolism of glucose. ChREBP is translocated between the cytoplasm and the nucleus. ChREBP enters the nucleus when glucose concentrations are high. Insulin concentrations are increased in the presence of high glucose concentrations and insulin will induce SREBP 1c transcription by a PI 3 kinase dependent mechanism. Synthesis of the precursor SREBP 1c occurs in the endoplasmic reticulum, and cleavage of the precursor occurs in the presence or absence of insulin. Mature SREBP 1c translocates to the nucleus to activate glucokinase and L-pyruvate kinase transcription.

ChREBP by regulating the entry of ChREBP into the nucleus and by activating the binding of the transcription factor to DNA. Glucose stimulates ChREBP to bind to the promoter of liver-type pyruvate kinase (L-PK), a key regulatory enzyme in glycolysis. L-PK catalyses the conversion of phosphoenolpyruvate to pyruvate (Figure 3), which enters Krebs cycle to generate citrate, a further source of acetyl-CoA used for fatty acid synthesis [23].

Recently, generation of ChREBP knockout mice showed that not only was the expression of L-PK reduced by approximately 90%, but also the mRNA levels of all fatty acid synthesis enzymes were reduced by approximately 50% [23,24]. This evidence suggests that ChREBP can independently stimulate the transcription of all lipogenic genes. Thus, activation of L-PK stimulates both glycolysis and lipogenesis, thereby facilitating the conversion of glucose to fatty acids under conditions of energy excess. The potential favourable effects of ChREBP activation on glucose catabolism may, therefore, have the disadvantage of inducing fatty liver [23,24]. Currently there is no pharmacological inhibitor of ChREBP, but it has been shown that troglitazone induces ChREBP gene expression in a dose-dependent manner. Whether modulation of the action of SREBP-1c results in inactivation of ChREBP and attenuates the development of NAFLD in insulin-resistant states is currently unknown.

Interestingly, liver X receptors (LXR) and in particular LXRα has been shown to induce transcription of the SREBP-1c gene. LXRs are members of the nuclear receptor family of transcription factors, and are recognised as important regulators of cholesterol metabolism, lipid biosynthesis and glucose homeostasis. An LXR agonist has been shown to improve glucose metabolism and also activate SREBP-1c resulting in severe fatty liver [25]. In summary, different factors are directly or indirectly involved in the regulation of the action of the SREBP-1c, for example glucagon, LXR, ChREBP, leptin and insulin. Whether suppression of the action of SREBP-1c results in modulation of these factors and unfavourable effects on plasma glucose or plasma triglyceride levels is uncertain. Whether selective pharmacological manipulation of specific inter-

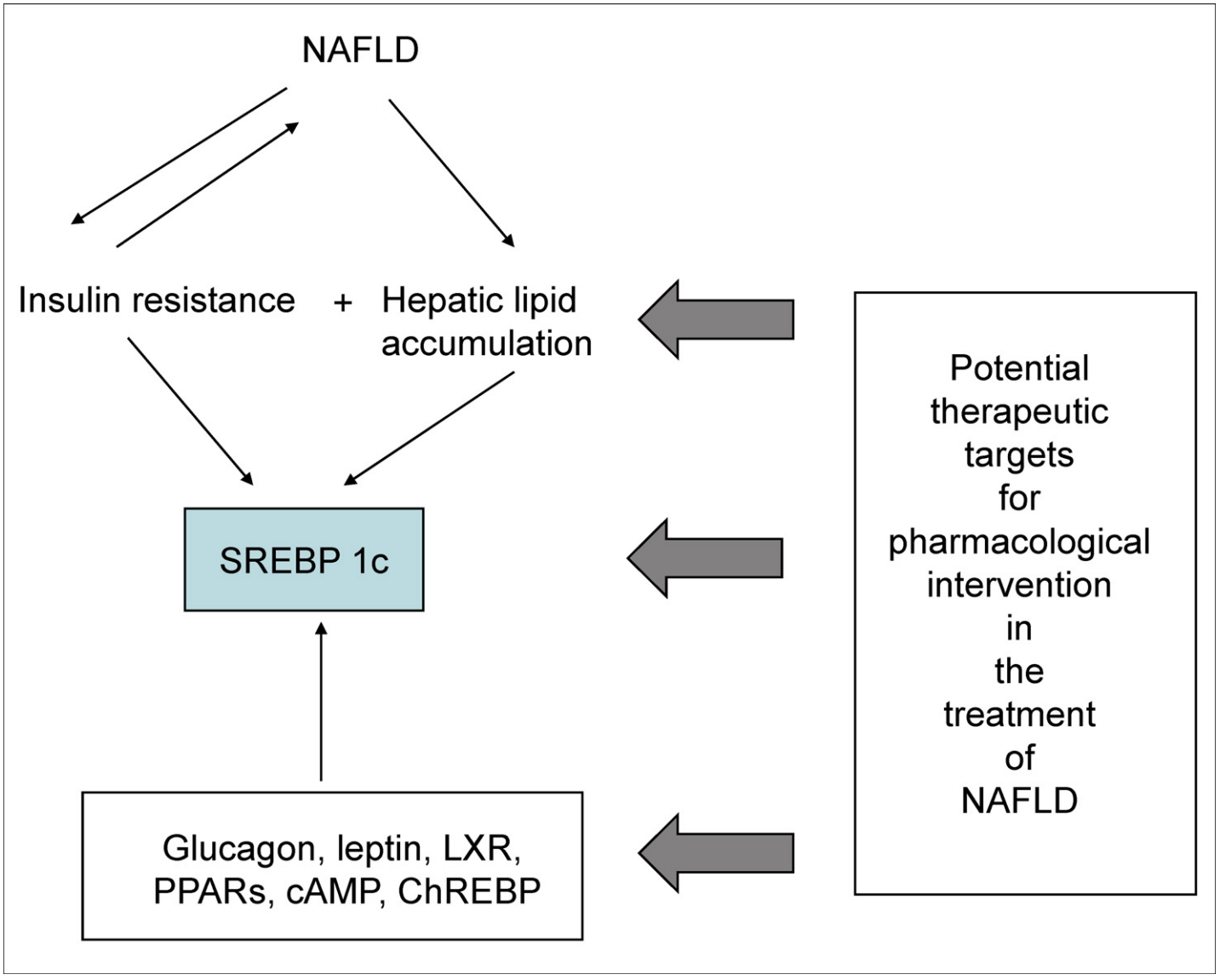

FIGURE 4

Potential therapeutic targets for treating NAFLD. The schematic figure shows the relationship between NAFLD insulin resistance and SREBP 1c and highlights key potential regulators of SREBP1c that may be amenable to therapeutic manipulation in the treatment of NAFLD.

actions between SREBPs and each of the above molecules is possible, now needs to be determined.

SREBPs and lipid homeostasis

SREBPs play a crucial role in maintaining normal physiology: SREBPs regulate expression of the LDL receptor that enables hepatocytes to remove cholesterol contained in LDL particles. High (dietary) cholesterol prevents maturation of SREBPs and not only decreases cholesterol synthesis but also LDL receptor synthesis, resulting in high plasma cholesterol and a predisposition to atherosclerotic plaque formation [8–12].

SREBP-activated genes predominantly belong to lipid synthetic pathways that include cholesterol synthesis, fatty acid synthesis, lipogenesis, triglyceride and phospholipid synthesis. Yet, the three SREBPs do not activate identical gene pathways. Using transgenic mice overexpressing just one type of SREBP, it has been shown that SREBP-1c actions favour fatty acid synthesis, whereas SREBP-2 action favours cholesterol synthesis, which may increase as much as 28-fold in the liver. SREBP-1a appears to be constitutively expressed in most tissues, with as yet no known factor that stimulates its expression [8–12]. Overexpression of SREBP-1c in adult rats also resulted in overstimulation of lipid synthesis, but in this case fatty acid synthesis was increased 26-fold, and cholesterol synthesis was increased 5-fold. SREBP-1c is predominantly involved in the regulation of adipogenesis and also in the regulation of insulin-responsive genes, which control lipogenesis and glucose metabolism [26]. SREBP-1c mRNA synthesis responds to nutritional changes in parallel with changes in insulin levels [10]. Insulin increases the expression of SREBP-1c and SREBP-regulated genes involved in the synthesis of saturated and unsaturated fatty acids [27,28] and glucose metabolism [10,18]. This effect is opposed by glucagon and cyclic AMP. The current evidence suggests that SREBP-1c mediates all effects of insulin on lipogenesis. Therefore, it is tempting to conclude that the SREBP-related links between fatty acid and glucose metabolism are also relevant to type 2 diabetes and obesity. It would appear that fatty liver frequently observed in diabetes could be the result of high SREBP-1c levels caused by increased insulin levels. In response to nutritional stimuli, for example carbohydrate in the diet, SREBP-1c also triggers expression of genes for enzymes regulating fatty acid elongation [29] and glycerol 3-phosphate acyltransferase required for triglyceride and phospholipid synthesis.

Ligands of the peroxisome proliferator activated receptor-γ (PPAR-γ) (two sub types α, δ) promote adipogenesis, stimulate glucose disposal in skeletal muscle and depress glucose production from the liver. The molecular mechanisms and target tissues through which the PPAR ligands (such as the thiazolidinediones) exert their antidiabetic activity are complex and beyond the scope of this review, but this activity is thought to have a direct effect in adipose tissue as well as insulin sensitivity. Insulin-induced gene (INSIG-1) is one of the genes induced in the adipose tissue of *db*/*db* mice treated with rosiglitazone (PPAR-γ ligand). INSIG-1 is also expressed in the liver and was also found to be induced both in epididymal fat in a diet-induced obesity model [30–32]. Li *et al.* [30] have proposed that INSIG-1 serves as a 'brake' for lipogenesis in fat, limiting the deposition of triglycerides in individual cells

TABLE 1

Summary of current potential treatment for NAFLD

Treatment	Evidence	Effect on liver
Caloric restriction and weight loss	Decreased insulin resistance	Decreased steatohepatitis
PPAR-γ agonist (for example, rosiglitazone, troglitazone)	Increased insulin sensitivity, decrease liver fat content	Improved histology and reduce inflammation
Biguanides example, metformin	Increased hepatic insulin sensitivity, increase AMP kinase activity	Reduced liver enzyme but no histological improvement
PPAR-α clofibrate, gemfibrozil	PPAR-α agonists	Increased hepatic fat oxidation
Statins		Associated with histological improvement
Miscellananeous (Ursodeoxycholic acid, Betaine, *N*-acetylcysteine, Vit E)	Decreased hepatic injury	Decreased free radicals and inflammation

and possibly also influencing the recruitment of preadipocytes. Furthermore, it has been suggested that regulation of INSIG-1 by PPAR-γ agonists could in turn regulate SREBP processing and thus couple insulin sensitizers with regulation of lipid homeostasis. Recently, administration of pioglitazone (a PPAR-γ agonist) led to metabolic and histological improvement in the first randomised placebo-controlled trial of pioglitazone in subjects with NASH [6].

PPAR α is predominantly expressed in the liver and is involved in promoting gluconeogenesis and regulating genes crucial for regulation of mitochondrial fatty acid oxidation. The fibrate class of drugs acts as PPAR-α agonists, decreases plasma triglyceride and increases HDL-c levels. It has been suggested that PPAR-α activation can suppress the activation of SREBP-1c [33,34]. It is clear from the above discussion that SREBP-1c plays a unique role in the homeostasis of hepatic lipid and glucose metabolism. Importantly, SREBP-1c may have a major role in the pathogenesis of fatty liver. Pharmacological manipulation of the SREBP system may prove beneficial in the management of fatty liver (see Figure 4). However, as SREBP-1c has both lipogenic and glucose-lowering effects, any kind of pharmacological manipulation will have to be carefully monitored for any undesired side effects. This again emphasises the need for selective agents that are able to modulate individual actions of SREBP-1c.

SREBPs and non-alcoholic fatty liver disease

Overexpression of SREBP-1c in the liver of transgenic mice produces a triglyceride-enriched fatty liver with no increase in cholesterol [8,9]. In rat hepatocytes, insulin treatment increases the amount of SREBP-1c [9]. Furthermore, SREBP-1c levels are increased in the fatty livers of obese (*ob*/*ob*) mice with insulin resistance and hyperinsulinaemia caused by leptin deficiency. The elevated SREBP-1c increases lipogenic gene expression, enhances fatty acid synthesis and accelerates triglyceride accumulation [8]. In addition, SREBP-1c appears to play a crucial role in the regulation of hepatic fats and hepatic glucose metabolism relevant to NAFLD. Figure 4 summarises the therapeutic targets and regulation of SREBP-1c that could form a basis for treatment of NAFLD in the future. However at present, different medications acting by different mechanisms have been suggested as potential therapeutic agents in the treatment of NAFLD. Interestingly, some of these medications have been shown to act directly or indirectly to modulate activity of SREBPs (Table 1).

Conclusion

NAFLD is strongly linked to obesity and insulin resistance that is associated with increased intra-hepatic production of free fatty acids from glucose not taken up by peripheral adipocytes and myocytes. Under the stress of increased free fatty acid flux to the liver, excess fatty acids are converted to triglycerides and stored in the cytoplasm, leading to steatosis. Insulin resistance affecting activity of the SREBP-1 c further increases hepatic steatosis. It is evident that SREBP-1c plays a unique role in the homeostasis of hepatic lipid and glucose metabolism. Importantly, SREBP-1c may have a major role in the pathogenesis of NAFLD, and we suggest that pharmacological manipulation of SREBPs may prove beneficial in the management of NAFLD. One potential therapeutic problem to overcome is that SREPB-1c has both lipogenic and glucose-lowering effects. Thus, any kind of pharmacological manipulation would need to be carefully developed to decrease this potentially undesirable effect. Interestingly, this effect could be therapeutically advantageous in people with type 2 diabetes, in whom NAFLD occurs frequently. At present, the corner stone of treatment for NAFLD is caloric restriction and gradual weight loss, to introduce a net negative energy balance and decrease liver fat. Increasing evidence is accumulating to suggest that PPARγ agonists may also be beneficial, but these agents are not licensed for treatment of NAFLD. We suggest that now the time is right to determine whether pharmacological agents capable of modulating SREBPs confer benefit in the treatment of NAFLD.

Conflicts of interest

CDB and MHA declare no conflicts of interest.

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