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REVIEW

Non-alcoholic fatty liver disease and cardiovascular risk: metabolic aspects and novel treatments

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Abstract Non-alcoholic fatty liver disease (NAFLD) is usually a silent disease that occurs in a very high proportion of people with features of the metabolic syndrome, including overweight, insulin resistance and type 2 diabetes. Because obesity and type 2 diabetes are now extremely common in Westernised societies, it is likely that the prevalence of NAFLD increases markedly in the future. Although previously it was thought that NAFLD was harmless, it is now recognised that NAFLD can be a progressive liver condition that increases risk of cirrhosis, end-stage liver disease and hepatocellular carcinoma. Additionally, liver fat accumulation causes insulin resistance and increases risk of type 2 diabetes. Increasing evidence now shows NAFLD is a risk factor for cardiovascular disease (CVD). The purpose of this review is to briefly discuss the pathogenesis of NAFLD, to describe the relationship between NAFLD and CVD and the mechanisms linking both conditions and to discuss some of the treatment options (including lifestyle, nutrition and drugs) that may influence both NAFLD and risk of CVD.

Keywords Non-alcoholic fatty liver disease (NAFLD) · Non-alcoholic steatohepatitis (NASH) · Cardiovascular disease (CVD) · Metabolic syndrome · Inflammation · Non-esterified fatty acids

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Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CVD	Cardiovascular disease
HDL	High-density lipoprotein
IL	Interleukin
IR	Insulin resistance
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NEFA	Non-esterified fatty acid ('free fatty acid')
NF-κB	Nuclear factor κB
RAS	Renin angiotensin system
TNF-α	Tumour necrosis factor α
TG	Triglyceride
VLDL	Very low density lipoprotein

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as a pathologic condition characterized by the deposition of triglyceride (TG) in the liver >5% of the total liver weight, in the presence of alcohol consumption <10 g daily [1]. The term NAFLD comprises a spectrum of liver disease. The first stage in the disease process is liver-fat accumulation or steatosis, and the second stage is non-alcoholic steatohepatitis (NASH) characterized by hepatocyte injury and inflammation. NASH is a progressive form of fatty liver that can worsen over time to end-stage cirrhosis and liver failure. In some patients with NASH or who have developed cirrhosis, it is now clear that a third stage may develop with the formation of a hepatocellular carcinoma and this can occur in a cirrhotic liver or even in NASH

without cirrhosis [2]. In Western Countries, NAFLD is found in about 20–30% of the general population, rising to 90% amongst obese subjects [3]. By contrast, in Eastern countries and in Italy, NAFLD occurs in about 12–24% [4] and 25% of the population, respectively [5].

A liver biopsy and histological diagnosis using Kleiner's histological NAFLD activity score is the gold standard to ascertain the stage of the disease [6]. The invasive nature of liver biopsy inevitably limits this diagnosis; consequently, non-invasive methods have been developed to diagnose NAFLD. Such methods include age, anthropometric measurements (body mass index) and biochemical tests (glycemia, platelet count, albumin and serum AST/ALT) [7, 8] in combination with ultrasound, CT-scan and magnetic resonance imaging (MRI) [9].

Recently, it has been theorised that NAFLD is the hepatic manifestation of the metabolic syndrome: a complex syndrome of inter-related metabolic disorders involving dyslipidaemia, visceral obesity, insulin resistance (IR), type 2 diabetes and hypertension [10]. The clinical manifestations of NAFLD, such as steatosis (stage 1) and inflammation (stage 2), are additional risk factors of cardiovascular disease (CVD) [11], although the precise mechanisms by which NAFLD contributes to CVD are still the subject of ongoing research.

Pathogenesis of NAFLD

Hepatic fat accumulation is a consequence of an imbalance between the accumulation and catabolism of TG in the liver, and schematic Fig. 1 illustrates the metabolic and pathophysiological processes contributing to the NAFLD and potential links between NAFLD and CVD. There are three sources of metabolites for the hepatic synthesis of TG: (1) dietary supply of fatty acids and glucose, (2) de novo synthesis of fatty acids and (3) adipose tissue supply of fatty acids from lipolysis.

Dietary fat is absorbed in the intestine, assembled into chylomicrons and released into the systemic circulation. About 80% of chylomicrons are hydrolyzed by lipoprotein lipase liberating the constituent fatty acids, and the remaining remnant is delivered to the liver [12]. De novo lipogenesis also contributes to VLDL assembly and de novo synthesis may contribute between 2 and 5% of VLDL-TG in healthy people [13] and 20–30% in pathophysiological states [14, 15]. Adipose tissue is the major source of non-esterified fatty acids (NEFAs) which accrue in the liver and are responsible for approximately 60% of TG accumulation [15]. The high flow of NEFAs to the liver causes a decrease of mitochondrial oxidation and a reduction of TG secretion into the systemic circulation as VLDL [16]. This mechanism contributes to hepatic TG

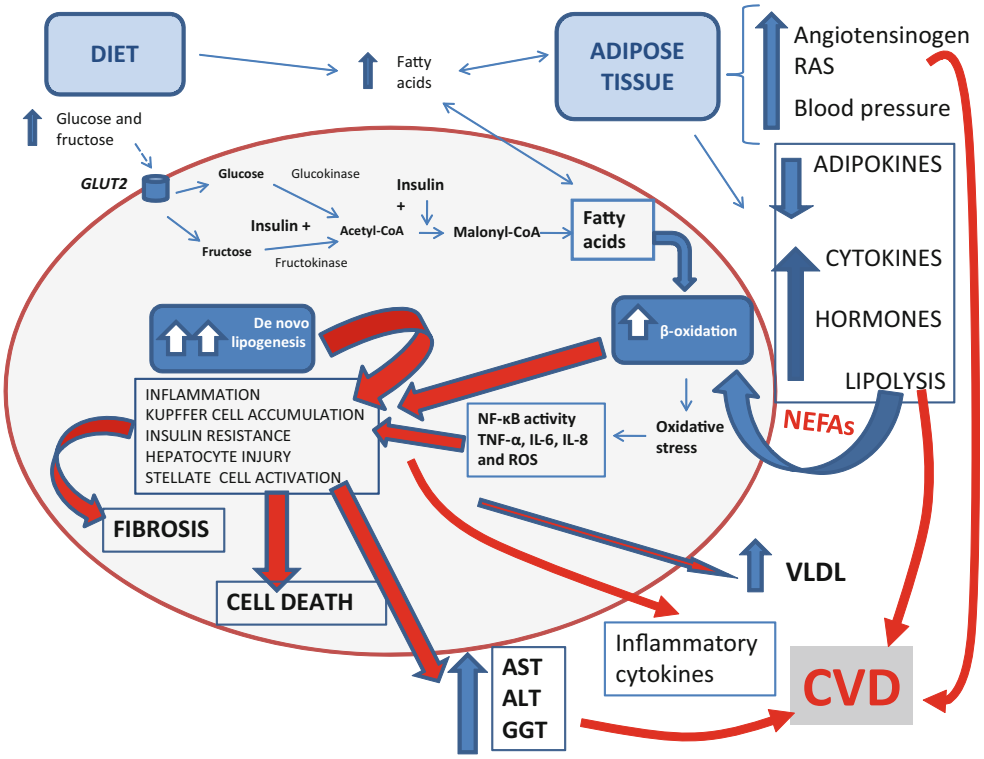

Fig. 1 Mechanisms linking non-alcoholic fatty liver disease (NAFLD) to cardiovascular disease (CVD). Schematic figure showing potential pathogenetic mechanisms linking dietary fat and carbohydrate intake with NAFLD, increased adiposity, blood pressure and CVD

storage resulting in liver steatosis and perhaps driving inflammation [17].

Contribution of diet to pathogenesis of NAFLD

Starting in the 1990s, the total calorie intake has increased and the physical activity levels have declined. The result is the beginning of the 'fifth phase of the epidemiologic transition: the *age of inactivity and obesity*' [18]. The adverse health consequence of this age is overweight and obesity, lipid abnormalities, type 2 diabetes mellitus and hypertension [19]. In ancient Greek medicine, diet was a central element of the *dìaita* or 'way of life'. Claudius Galenus (A.D. 129–199) in his '*De alimentorum facultatibus*' (On the Nature of Foods) considered food and physical activity two 'non-natural' aspects of life, classifying them as cultural and not inherent to the corporeal humours; furthermore, he believed that these non-natural aspects could be manipulated by human beings to preserve health [20]. If the diet is unbalanced, the intake of certain nutrients may be inadequate or excessive and nutrients may be consumed in the wrong proportions to one another, leading to a status of *malnutrition.* At present, Western-style diets are characterized by high intake of calories, resulting in increased body weight, obesity and chronic diseases; this type of diet frequently contains excessive carbohydrates, including starches and sugars, and saturated and *trans* fatty acids and has too little omega-3 polyunsaturated fatty acids (*n*-3 PUFAs) [21].

Carbohydrates

High-carbohydrate diets increase hepatic de novo lipogenesis [22]. This is a multi-stage process in which (i) dietary glucose is transported into the liver by glucose transporter-2 (GLUT-2), (ii) dietary glucose is phosphorylated by the enzyme glucokinase and converted by liver-specific pyruvate kinase into acetyl-CoA, (iii) acetyl-CoA is converted into malonyl-CoA in the presence of insulin (that activates acetyl-CoA carboxylase) and (iv) malonyl-CoA is the committed substrate on the fatty acid synthesis pathway. The activity of the glycolytic and lipogenic enzymes is controlled at transcriptional level by SREBP-1c (sterol regulatory element binding protein-1-c) and ChREBP (carbohydrate response element binding protein). SREBP-1c and ChREBP are up-regulated in conditions of hyperinsulinaemia and hyperglycaemia, respectively.

The effect of fast-food-based hyper-alimentation on liver enzymes and hepatic TG has been studied in 18 young healthy volunteers. After 4 weeks, body weight increased 5–15%, hepatic TG content TG rose 2.5-fold and there was an increase in serum ALT levels [23]. Furthermore, in support of the importance of body fat accumulation to affect lipid metabolism, weight loss of 10% in women with abdominal obesity decreased the fasting VLDL TG secretion by 40% [24]. Interestingly, in obese subjects, serum levels of ALT, TG and day-long insulin concentration decreased markedly with a low-carbohydrate diet (40% carbohydrate, 45% fat), compared with a traditional low-fat diet (60% carbohydrate, 25% fat) [25].

The change in the diet alluded to earlier has also included increased consumption of soft drinks containing fructose. In the liver, fructose is taken up by hepatocytes by the glucose transporter (GLUT-2) and subsequently phosphorylated to fructose-1-phosphate by the specific enzyme fructokinase. Fructose-1-phosphate is metabolized to glyceraldehyde-3-phosphate by aldolase B; thereafter, glyceraldehyde-3-phosphate is converted into pyruvate and subsequently into acetyl-CoA. Interestingly, fructose metabolism markedly differs from that of glucose as it is insulin independent and is not regulated by the levels of ATP and citrate. Fructose also promotes the expression of the transcriptional factors SREBP-1 and ChREBP, involved in the regulation of de novo lipogenesis, and also increases plasma uric acid concentration [26, 27]. Recent studies have also investigated the effect of fructose on intestinal bacteria. In the intestine, fructose is absorbed by a specific transporter located at the apical side of the enterocyte: glucose transporter-5. Chronic high intake of fructose may cause overgrowth of intestinal bacteria and increase intestinal permeability, leading to a translocation of bacterial endotoxin into the bloodstream, which in turn activates Kupffer cells, ultimately causing inflammation and accumulation of fat in the liver [28]. The effect of soft drinks on metabolic risk factors has been evaluated in healthy middle-age adults whose daily soft drink consumption was collected through a questionnaire. A high occurrence of metabolic syndrome was observed in those who consumed one or more soft drinks per day; furthermore, it was also found that the same subjects had a high incidence of increased waist circumference and hypertriglyceridemia [29]. In the Nurses' Health Study, a significant positive association between soft drinks consumption and risk of coronary heart disease was observed. The consumption of $\geq$2 soft drinks per day was related to an RR of 1.39 (95% CI: 1.11–1.75; $P < 0.001$), compared with those who consumed <1 soft drink per month [30]. Moreover, daily consumption of soft drinks ($\geq$7 servings per week) was associated to a progression of NAFLD into steatosis through the lipogenic and pro-inflammatory effect of fructose [31].

Lipids

A prolonged period of imbalanced intake of nutrients and the consequent accumulation of energy lead to a state of

allosteric overload that may cause obesity, IR, metabolic syndrome features and diabetes (Fig. 2) [32]. With a twenty-first century lifestyle that includes physical inactivity and excess calorie intake, there is a surplus of fat accumulated in ectopic visceral sites in insulin resistant individuals. For example, with insulin resistance, fat accumulates in visceral organs and tissues, such as skeletal muscle myocytes, hepatocytes and β-cells, and accumulation of fat in insulin-sensitive tissues (such as liver and muscle) tends to impair insulin signalling in these tissues. Research has demonstrated that there is not a direct correlation between adipose tissue total mass and insulin resistance [33], but a recent study showed that a genetically modified mouse (adiponectin-TG ob/ob) with twice the adipose tissue mass of a normal mouse had an optimum distribution of lipid in adipose tissue, leading to the hypothesis that a substantial increase in the capacity for adipose tissue to expand may prevent the development of IR and reduce lipid flow to non-adipose tissue organs and thereby limit ectopic fat accumulation [34]. Insulin-sensitive individuals tend to have the capacity to easily expand adipose tissue depots in subcutaneous adipose tissue depots in the presence of a twenty-first century lifestyle, thereby protecting their visceral organs from ectopic fat accumulation Fig 3.

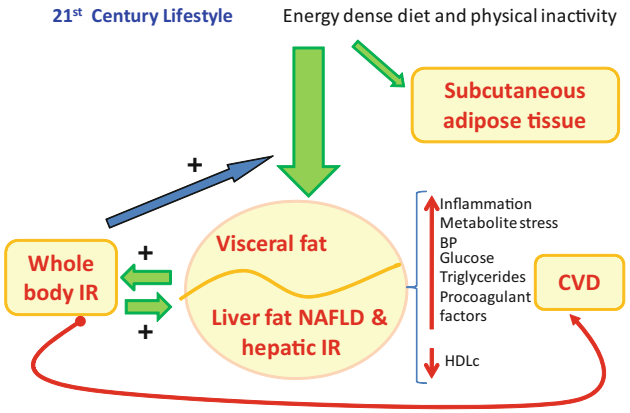

Fig. 2 Relationships between twenty-first century lifestyle, non-alcoholic fatty liver disease (NAFLD), whole body insulin resistance and cardiovascular disease (CVD). A twenty-first century lifestyle is associated with physical inactivity and excess dietary carbohydrate and fat intake. In the presence of whole body insulin resistance, there is a predisposition towards storage of excess dietary calories as triglyceride in ectopic visceral sites, rather than peripheral subcutaneous adipose tissue depots. Accumulation of fat in ectopic visceral tissues such as the liver exacerbates insulin resistance in hepatic tissue, compounding the problem and creating a positive feedback loop driven by continuing physical inactivity and excess dietary carbohydrate and fat intake. The development of NAFLD may contribute to development of CVD through a variety of mechanisms that include increased inflammatory and metabolic stress, disturbances of triglyceride-rich lipoprotein metabolism (that causes decreased high-density lipoprotein cholesterol concentrations) and release of pro-coagulant factors

In the presence of IR, adipose tissue hormone-sensitive lipase fails to be regulated, causing uncontrolled lipolysis and thus an increased flow of NEFAs to the liver. In addition, in this scenario, there is increased hepatic gluconeogenesis in insulin-resistant states with diversion of glucose to the hepatic lipogenesis pathway which further increases the accumulation of fat in the liver. Excessive storage of fatty acids in hepatocytes and insulin resistance may increase mitochondrial β-oxidation free-radical production and the production of reactive oxygen species may induce NF-κB activity, leading to a production of pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-8. The increase in oxidative stress may cause mitochondrial damage and dysfunction, leading to a decrease of oxidative capacity, which in turn may produce an imbalance between fat oxidation and lipogenesis pathways, ultimately resulting in liver-fat accumulation and NAFLD [35].

Trans-fatty acids

Throughout the twentieth century, the hydrogenation of fatty acids became an increasingly common practice in food processing; this process can result in a change of double-bond configuration in fatty acids from *cis* to *trans* to increase their stability for food longevity. *Trans*-fatty acids have similar physical and physiological properties to saturated fatty acids and they are similarly associated to CV risk factors, as they increase plasma total and low-density lipoprotein cholesterol concentrations [36]. Unsurprisingly, *trans*-fatty acids consumption is associated with increased weight gain and intra-abdominal fat distribution [37]; nevertheless, there is no strong evidence of an association with the development of IR. Further studies are necessary to elucidate the role of *trans*-fatty acids in NAFLD.

Choline

A low-choline diet (the recommended daily intake of choline is 550 mg/day for men and 425 mg/day for women) [38] has also been associated with NAFLD and CV disease [39]. Choline plays an important role in the production of phosphatidylcholine (PC), the predominant phospholipid in mammalian cell membranes. PC is synthesized in the hepatic endoplasmic reticulum; 70% originates from the dietary-dependent pathway (dietary choline pathway) and the remaining 30% from the dietary-independent pathway (phosphatidylethanolamine *N*-methyltransferase: PEMT pathway). Biosynthesis of PC by both pathways is fundamental for the assembly and secretion of VLDL and bile from the liver and 50% of PC secretion from the liver is in bile [40]. Mice on a choline-deficient diet and lacking PEMT develop severe steatohepatitis and liver bile accumulation after 3 days; whereas by contrast,

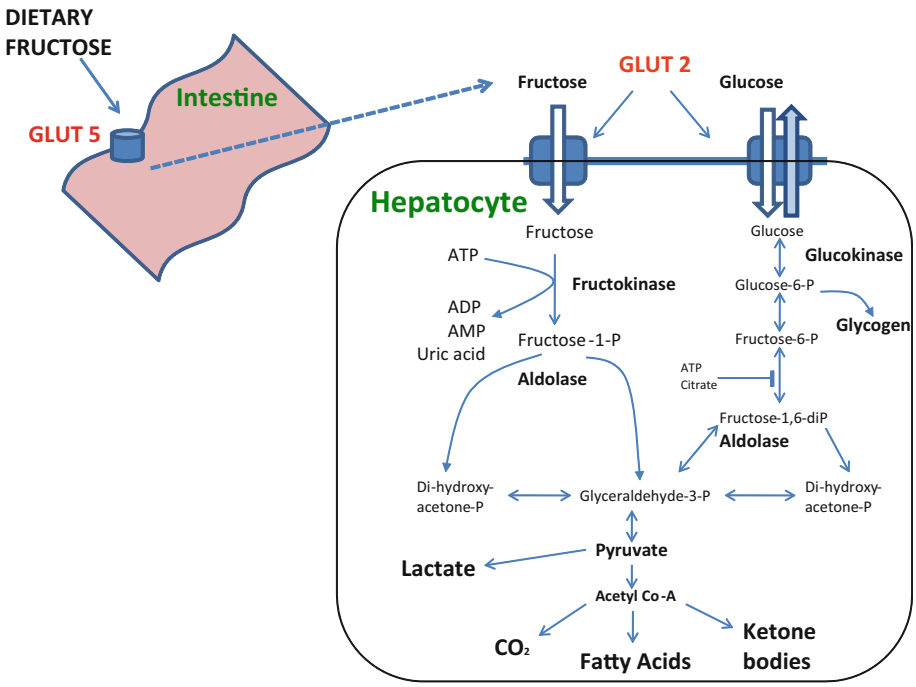

Fig. 3 Mechanism linking dietary fructose intake and hepatic fatty acid metabolism. Fructose is transported into enterocyte by the specific fructose transporter GLUT 5. In the liver, fructose metabolism is independent of insulin action and is also not regulated by the levels of ATP and citrate. Dietary fructose intake increases intrahepatic fructose-1-P and thereby increases uric acid formation. Fructose-1-P can be converted into glyceraldehyde-3-phosphate and thereby into acetyl Co-A (the precursor for fatty acid synthesis). Thus, dietary fructose intake may stimulate hepatic de novo lipogenesis and thus potentially be involved in the pathogenesis of NAFLD

mice on a choline-deficient diet, that have active PEMT, do not [41]. This evidence shows that the PEMT pathway has a fundamental role in producing PC if the dietary intake of choline is insufficient. However, mice lacking the PEMT pathway are protected against diet-induced obesity and IR [42]. Nevertheless, the PEMT reaction synthesizing one molecule of PC from phosphatidylethanolamine generates three molecules of *S*-adenosylhomocysteine (SAH) that are hydrolyzed in the liver to homocysteine. Crucially, an increase in the plasma level of homocysteine is an independent risk factor of atherosclerosis, CV disease and stroke [43, 44]. Therefore, the consequence of dietary-choline depletion is increased activity of the PEMT pathway that leads to increased plasma homocysteine and there is now evidence that disrupting function of the PEMT gene in mice (fed a high-fat/high-cholesterol diet) decreases plasma TG and reduces plasma cholesterol levels by 20–40%. Furthermore, and interestingly, PEMT deficiency decreases homocysteine and atherosclerotic lesions by 80% [45] and more research is needed to study the role of the PEMT pathway in NAFLD.

There is also evidence that changes in gut microflora can influence choline metabolism and thereby influence development of NAFLD. For example, in 10% of gut bacteria, PC is an important component of the bacterial cell wall and components of the diet can affect choline metabolism and alter gut bacterial wall composition [46]. Dietary methylamines can cause choline hydrolysis and thereby modify gut bacterial cell walls affecting the normal healthy and pathogenic colonic microflora. These effects could be potentially important for the pathogenesis of NAFLD and CVD as changes in gut microflora have been linked to fatty liver disease [46, 47] and CVD [48].

Metabolic aspects of NAFLD and relation to CVD

Oxidative stress and inflammation

Patients with NAFLD generally present with the symptoms and signs of the metabolic syndrome (central obesity, hypertension, dyslipidemia and hyperglycemia) and therefore NAFLD is associated with multiple potential risk factors for CVD [49, 50]. It has now been shown that NAFLD is an independent risk factor for death from CVD [51] and although the precise mechanism(s) linking NAFLD and CVD is unclear, a systematic review and meta-analysis have shown that a marker of NAFLD (and oxidative stress) may be the key link between NAFLD and CVD [52]. These data suggest that some component of oxidative stress, perhaps induced by the disease process in NAFLD, may be involved in the pathogenesis of CVD.

Visceral adipose tissue is a metabolically active endocrine organ that can secrete pro-inflammatory cytokines, adipokines and hormones causing inflammation and IR that, in turn, affect CV risk factors [53, 54]. Insulin resistance and excessive fat storage in visceral adipose tissue cause increased flow of NEFAs to hepatocytes, resulting in increased β-oxidation and oxidative stress. Increased mitochondrial fat oxidation produces reactive oxygen species (ROS) and upregulates the nuclear factor kappa-B (NF-κB) that activates the transcription of

several pro-inflammatory genes. This mechanism leads to production of the pro-inflammatory cytokines (TNF-α, IL-6 and IL-8) by hepatocytes, Kupffer cells and hepatic stellate cells [35, 55]; in patients with NASH, there is a high level of inflammatory markers, pro-coagulant factors and oxidative stress markers [56]. Chronic inflammation originating from adipose-derived factors and hepatic activation of the NF-κB pathway may cause further hepatocellular damage, leading to progression of liver disease and further risk of CVD [57, 58].

Treatment

Lifestyle modifications, such as increased physical activity, positively affect nutritional behaviour, leading to a gradual weight loss, which in turn improves metabolic syndrome, reduces CV risk and decreases NAFLD. However, the effects of lifestyle change are not instantaneous, and weight loss should be a gradual process. Moreover, weight loss and increases in physical activity are often difficult to achieve and consequently there is a need for novel pharmacological treatments that are capable of controlling risk factors that contribute to NAFLD and CVD.

Lifestyle modification: weight loss, physical activity and pro- and prebiotic treatment

Many studies have shown the beneficial effects of lifestyle modifications in subjects who are overweight with metabolic syndrome, CVD and NAFLD (summarized in Table 1) [59–63]. Dietary intervention and physical activity, however, require intensive efforts to attain significant improvements in CV risk factors and liver histology. Therefore, achieving and maintaining weight loss solely by virtue of lifestyle modifications is challenging. These difficulties can be overcome by adopting a multidisciplinary approach that involves physicians, dieticians, physical activity specialists and psychologists whose combined effort is better suited to tackle the behaviour and cognitive components underlying lifestyle modifications [64].

A new therapeutic approach that requires further testing in NAFLD is treatment with probiotic and prebiotic agents. Pro- and prebiotic agents modify intestinal microbiota and reduce intestinal permeability and these effects may improve insulin sensitivity and NAFLD histology [65].

Drugs

n-3 PUFAs

As previously stated, a Western diet contains limited amounts of *n*-3 PUFAs and interestingly, these particular fatty acids may play an important role in preventing and improving NAFLD. For example, *n*-3 PUFAs (i) reduce hepatic fatty acid content through decreasing hepatic de novo lipogenesis by activation of peroxisome proliferator-activated receptor alpha (PPAR-α); (ii) downregulate SREBP-1c, which is the key transcriptional factor of de novo lipogenesis; (iii) improve IR [66]; (iv) diminish inflammation by reducing activation of NF-κB; and (v) lower plasma TG and ALT [67]. A therapeutic dose of 2–3 g of *n*-3 PUFAs decreases plasma levels of TG by 30%, and although there is a theoretical benefit for the use of *n*-3 PUFAs in NAFLD, and there is some evidence of benefit in treating atherosclerosis [68], more evidence is required to test the effects of *n*-3 PUFA treatment in NAFLD.

Ezetimibe

Ezetimibe binds to Niemann-Pick C1-like 1 (NPC1L1) intestinal cholesterol transporter, inhibiting intestinal cholesterol absorption. Several studies have proved the effectiveness of ezetimibe monotherapy (10 mg/day) in lowering low-density lipoprotein cholesterol (−18%), improving high-density lipoprotein cholesterol (3%), reducing TG concentrations (−8%) and decreasing total cholesterol (−13%) when compared with a placebo [69]. Moreover, ezetimibe increases Apo-A-I, decreases Apo B levels [70] and reduces cholesterol absorption markers [71]. Furthermore, it has been shown that ezetimibe treatment was associated with a 40% decrease in plasma alanine aminotransferase (ALT) levels [72].

Recently, the beneficial effects produced by ezetimibe therapy in NAFLD have sparked some interest [73]. Liver cholesterol accumulation promotes production of TNF-α, through oxidative stress generated in mitochondria. Lipotoxicity leads to adipocyte hypertrophy and hypoxia, unfolded protein response and endoplasmic reticulum stress; these mechanisms activate inflammatory pathways and stimulate necrosis. All these factors contribute to IR, along with the secretion of fatty acid (within VLDL) to supply distant tissues, potentially increasing metabolic dysfunction and CV risk [74].

Blockade of the NPC1L1 cholesterol transporter may modulate intracellular cholesterol, reducing expression of SREBP-1c and ChREBP, which are key transcriptional regulators of hepatic lipogenic gene expression. Recent studies have shown that patients with NAFLD have significantly higher levels of palmitic, palmitoleic, oleic and γ-linoleic acids in plasma cholesteryl esters, higher estimated activity of delta-9 and delta-6 desaturases and lower estimated activity of delta-5 desaturase compared with normal controls. This composition of fatty acids is also a potential risk factor, as this metabolic disturbance has been

Table 1 Effect of lifestyle modification on risk factors for cardiovascular disease and non-alcoholic fatty liver disease

Study	*n*	Patients	Age (years ± SD)	Sex M/F %	BMI (mean ± SD)	Lifestyle intervention	Primary outcome measure	Finding
Ueno et al. [59]	25	Overweight with NAFLD	39 ± 13	52/48	31 ± 5	About 25 kcal decrease per kg of estimated ideal body weight combined with increased daily physical activity.	Histological grading; serum aminotranferases	About 10% reduction in BMI, decreased hepatic steatosis and decreased serum ALT and AST
St George et al. [89]	152	Central obesity with elevated liver enzymes	47.5 ± 12.4	38/18	31.9 ± 6.0	Three months restricted diet (daily diet low in saturated fats and processed food and high in omega 3 and fibre with reduction in daily intake of 406–573 kcal for weight loss) and moderate physical activity (150 min/week for general health and ≥200 min/week for weight loss)	Serum liver enzymes	Decrease in serum ALT, AST and γGT related with weight loss
Sreenivasa et al. [60]	94	NAFLD	38.7 ± 9.5	78/22	26.7 ± 3.8	Three months restricted diet (25 kcal per kg ideal body weight (IBW) from a diet containing 60% carbohydrate, 20% fat, 20% protein)	Serum aminotransferases; histological grading; lipid profile	About 4–4.5% reduction in weight and BMI and 50% decrease in ALT
Chainani-Wu et al. [61]	131	56 with CHD	58.2 ± 7.6	35/65	33.6 ± 6.9	Three months restricted diet (10% fat, 15% protein and 75% complex carbohydrates); 3 h each week of aerobic exercise with a minimum of 30 min per session and/or perceived exertion levels, to perform strength training activities a minimum of two times each week.	Low-density lipoprotein cholesterol; triglycerides; body mass index	Reduction in BMI, total cholesterol and LDL cholesterol
Lawlor et al. [62]	3,789	British Women's Heart and Health Study	60–79	−/100	30.4	About 4 years moderate physical activities (2–3 h/week)	Body mass index; waist:hip ratio; serum liver enzymes	Positive linear association between body mass index, waist:hip ratio and serum ALT and γGT

related to metabolic disease and CV risk. Long-term treatment with ezetimibe decreased the levels of myristic, palmitic, palmitoleic, oleic and dihomo-γ-linoleic acids and delta-9 desaturase activity; and in contrast, increased linoleic acid level and delta-5 desaturase activity. Moreover, in two studies by Park et al., it was demonstrated that ezetimibe monotherapy may improve liver histology according to steatosis grade, necroinflammatory grade, ballooning score and NAS score [75, 76].

The effect of ezetimibe on IR has also been examined in obese Zucker rats. Nomura et al. [77] have shown that blockade NPC1L1 by ezetimibe lowers hepatic steatosis and restores hepatic insulin sensitivity, suggesting important effects of ezetimibe to modify insulin action. Thus, the preliminary evidence suggests that there may be a beneficial effect of treatment with ezetimibe in people with NAFLD but clinical trials are now urgently needed to test the effects of this agent in patients who have the spectrum of NAFLD conditions.

Fibrates

Fibrates are drugs used for the management of hypertriglyceridaemia. This class of drugs is PPAR-α receptor agonists and affects regulation of carbohydrate and fat metabolism and adipose tissue differentiation. Fibrates decrease TG levels by 30–50% and increase HDL cholesterol by about 10–20%; however, they have little effect on LDL particles [78]. In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, it was shown that fenofibrate treatment produced a non-significant decrease in cardiovascular events [79] and more evidence is needed testing the effects of fibrates in NAFLD.

Statins

Statins are a good treatment for lowering cholesterol levels and reducing CV risk. Statins are a competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme in the cholesterol biosynthetic pathway, so prompting hepatic uptake of circulating LDL particles. However, there are numerous diverging studies concerning the safety of statins and their effect on liver tests. In the recent GREACE study, 1,600 patients with dyslipidemia were enrolled. The majority of the patients were obese and had abnormal liver tests or metabolic syndrome, with or without diabetes mellitus. NAFLD was diagnosed, by ultrasonographic diagnosis of the presence of liver fat, amongst those that had abnormal liver tests. At the end of the study, there was a significant reduction in total cholesterol, LDL cholesterol and TG compared with control subjects. Moreover, in patients with high concentrations of ALT, AST and GGT, there were reductions of these enzymes by 35, 47 and 46%, respectively [80]. Other studies have explored the possible clinical benefits of measurement of advanced glycation endproducts (AGEs) as biomarkers for NASH to test the effects of statins. Atorvastatin may lower the levels of these biomarkers that are also implicated in many age-related diseases. At the end of the study, liver biopsies showed that steatosis grade, necroinflammatory grade and NASH were improved significantly, although fibrosis stage was not changed. Serum levels of AGEs were significantly decreased after both 6 and 12 months of treatment with atorvastatin [81].

The adverse metabolic effect of statins in developing diabetes has recently been the subject of meta-analyses. In a meta-analysis of large randomized trials with atorvastatin, it was shown that high doses of atorvastatin were associated with increased risk of new-onset type 2 diabetes mellitus [82]. The small effect of atorvastatin to increase plasma glucose is probably not clinically relevant, given the marked potential benefit of atorvastatin to decrease cardiovascular events. Thus, recent evidence suggests that statins may have a small deleterious impact on glucose tolerance but this effect is not sufficient that it should influence prescribing of statins in people at high risk of vascular disease. Although recent evidence, therefore, suggests a potential benefit of statins on liver fat and possibly on liver inflammation, further work is needed to test the effects of statins in patients recruited because of NAFLD per se.

Drugs that improve insulin sensitivity and affect glucose tolerance

Other possible therapeutic approaches capable of ameliorating metabolic syndrome and reducing CV risk and NAFLD involve drugs that improve insulin sensitivity.

Metformin Metformin is a biguanide and is the first choice in oral treatment of type 2 diabetes. Metformin reduces hepatic glucose output, increases the insulin-mediated glucose use in peripheral tissues and improves body weight [83]. However, there is little evidence of an effect of metformin to ameliorate or retard disease progression in NAFLD.

Acarbose Acarbose is an inhibitor of alpha-glucosidase which reduces the absorption of dietary carbohydrate. Interestingly, combination therapy of acarbose and ezetimibe in a mouse model of liver fat reduced steatosis, inflammation and fibrosis in liver and decreased serum ALT level, when compared with ezetimibe monotherapy, acarbose monotherapy and a control group. Total cholesterol was decreased with both monotherapy and combination therapy and serum cholesterol–chylomicron levels

were significantly lower in the combination therapy group. Interestingly, cholesterol–VLDL was lower in the ezetimibe monotherapy group [84].

Thiazolidinediones Thiazolidinediones (TZDs) are another group of drugs used to decrease plasma glucose in type 2 diabetes. This class of drugs have recently fallen out of favour because of the problems with weight gain, fluid retention and increase risk of fracture. However, TZDs promote glucose and fatty acid uptake into adipocytes by activating PPAR-γ [85]. Pioglitazone has been shown to improve steatosis, ballooning necrosis and inflammation, but may have little effect on fibrosis in patients with NAFLD [86]. At present, more evidence of benefit is needed before pioglitazone can be recommended as a potential treatment for NAFLD.

Aleglitazar Aleglitazar is a new dual PPAR-α-γ agonist that improves glycemic control and lipid parameters. Aleglitazar decreases fasting plasma glucose, IR, glycosylated haemoglobin, TG, LDL-C and Apo B and increases HDL-c concentration. However, there are important side effects of therapy with aleglitazar and decreased renal function, increased CPK and plasma creatinine, weight gain and peripheral edema [87] will limit any potential use in NAFLD.

Renin-angiotensin system (RAS) blocker Renin-angiotensin system (RAS) blockers are another class of drugs that may improve insulin sensitivity, adipokine production, ALT levels and prevent hepatic stellate cell activation. Preliminary evidence suggests a benefit of treatment in NAFLD with a decrease in hepatic inflammation and fibrogenesis [88] and further studies are needed to test the effects of this class of drugs.

Conclusions

The clinic aspects of NAFLD are the consequence of metabolic disequilibrium between (i) glucose tolerance, (ii) provision of NEFAs to the liver from diet and adipose tissue and (iii) hepatic fatty acid production from glucose and fructose by de novo lipogenesis. This metabolic disequilibrium leads to the accumulation of fatty acid in the liver causing an increase in β-oxidation and oxidative stress that affect hepatic secretion of pro-inflammatory cytokines, pro-coagulant factors and triglyceride-rich lipoproteins. Each of these factors has the potential to affect the vasculature and promote atherogenesis. Importantly, further studies are urgently needed (a) to improve non-invasive methodology to diagnose and monitor NAFLD and (b) to investigate potential therapeutic options to affect both NAFLD and CVD.

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Disclosures The authors are undertaking the WELCOME study (Wessex Evaluation of fatty Liver and Cardiovascular markers in NAFLD [non-alcoholic fatty liver disease] with OMacor thErapy) in people with NAFLD. The WELCOME study is a phase IV trial that is testing the effects of high dose purified *n*-3 long chain fatty acids (Omacor–Solvay/Abbott/Pronova 4 grames o.d.) on a range of liver and cardio-metabolic outcomes. The trial will be completed in 2012 (www.clinicaltrials.gov registration number NCT00760513).

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