Obesity as the common soil of non-alcoholic fatty liver disease and diabetes: Role of adipokines

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) describes a spectrum of liver conditions from simple steatosis, steatohepatitis to end-stage liver disease. The prevalence of NAFLD has been on the rise in many parts of the world, including Asia, and NAFLD is now the liver disease associated with the highest mortality, consequent to the increased risk of cardiovascular diseases and hepatocellular carcinoma. Whereas NAFLD is an independent risk factor for type 2 diabetes, increased hepatic and peripheral insulin resistance contribute to the pathogenesis of both NAFLD and diabetes, which are associated with enhanced cardiovascular risk. Studies in humans and animal models have suggested obesity as the common link of these two diseases, likely mediated by adipose tissue inflammation and dysregulated adipokine production in obesity. In the present review, we discuss recent advances in our understanding of the role of several novel adipokines (adiponectin, adipocyte fatty acid binding protein and fibroblast growth factor-21) in the pathophysiology of NAFLD and diabetes, as well as their use as potential biomarkers and therapeutic targets for dysglycemia in NAFLD patients. (J Diabetes Invest, doi: 10.1111/jdi.12093, 2013)

KEY WORDS: Adipocyte fatty acid binding protein, Adiponectin, Fibroblast growth factor-21

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) was first recognized in 1980. Over the past few decades, it has rapidly become the most common form of liver disease, concomitant with the increasing prevalence of obesity worldwide[1]. NAFLD describes a spectrum of liver conditions ranging from simple steatosis to severe steatosis with marked inflammation, termed non-alcoholic steatohepatitis (NASH), which can be complicated by cirrhosis, end-stage liver failure and hepatocellular carcinoma[2,3]. Population screening has estimated the prevalence of NAFLD diagnosed on ultrasound (US-NAFLD) in the general population in Asian countries to be approximately 15–20%[4,5], akin to that in Western countries[6,7]. Its prevalence has doubled in urban Chinese cities in the past two decades[8,9]. People with NAFLD are usually asymptomatic at the early stage. However, NAFLD patients have a higher overall mortality than the general population[10,11]. In a 21-year follow up of biopsy-proven NAFLD, the main causes of death were cardiovascular disease and malignancy[12], as opposed to cirrhosis in those with alcoholic liver disease. The pivotal links between NAFLD and cardiovascular disease are metabolic disorders, including diabetes, dyslipidemia and hypertension[13,14].

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A strong association exists between NAFLD and type 2 diabetes, with NAFLD found in up to 70% of patients with type 2 diabetes[15]. In addition, a significant proportion of patients with NAFLD develop impaired glucose tolerance (IGT) or type 2 diabetes, dyslipidemia or hypertension a median of 6 years after diagnosis of NAFLD[16]. In a 5-year retrospective review, participants with US-NAFLD had higher risks of impaired fasting glucose, type 2 diabetes, insulin resistance and hypertriglyceridemia than NALFD-free controls[17]. Furthermore, the presence of type 2 diabetes is associated with a more progressive course and higher rate of progression to cirrhosis[18]. Thus, prediction and early intervention of dysglycemia in NAFLD might have additive benefits in reducing cardiovascular risk and decreasing the rate of NAFLD progression.

Obesity is a major risk factor of both NAFLD and type 2 diabetes, and likely provides the common link through insulin resistance (Figure 1). Specifically, visceral, liver and skeletal fat accumulations each play distinct, but overlapping roles in the development of insulin resistance. It is now recognised that insulin resistance in obesity is largely consequential to adipose tissue inflammation and adipokine dysregulation[19].

RELATIONSHIP BETWEEN LIVER, ADIPOSE TISSUE AND TOTAL INSULIN RESISTANCE

NAFLD and type 2 diabetes are associated with hepatic and adipose tissue insulin resistance, and reduced whole-body insulin sensitivity. The ability of insulin to suppress hepatic glucose production was impaired to a similar extent in subjects with

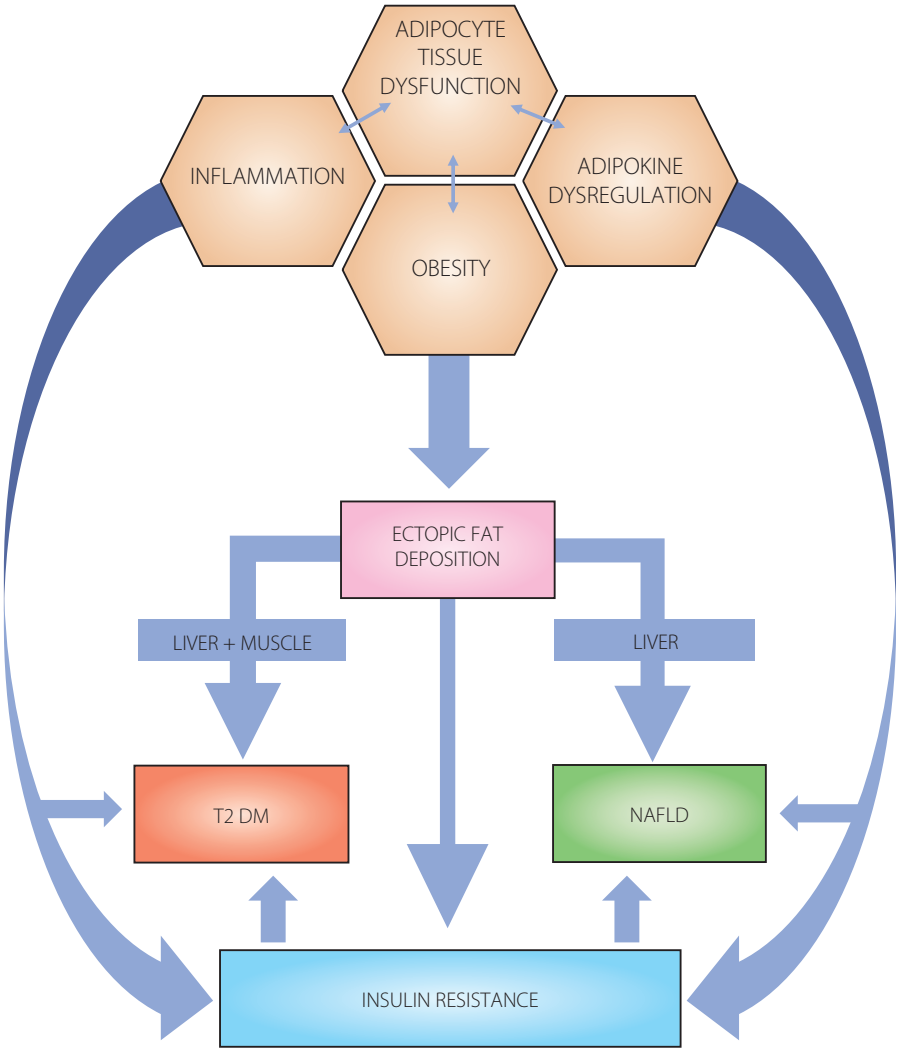

Figure 1 | Obesity is a common link between type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD). Adipose tissue dysfunction is characterized by inflammation and adipokine dysregulation, and subsequent ectopic fat deposition in the abdominal viscera and liver, and insulin resistance. It significantly contributes to the development of NAFLD and diabetes mellitus.

NAFLD and in those with type 2 diabetes. Glucose disposal during clamp study, a measure of whole-body insulin sensitivity, was reduced by nearly 50% in NAFLD subjects, similar to that in type 2 diabetes patients[20].

The pathogenesis of NAFLD was originally described by the 'two-hit hypothesis', and subsequently, modified as the 'multi-hit hypothesis', which describes the first hepatic insult as the dysregulation of fatty acid metabolism, leading to steatosis[21]. Insulin resistance plays a central role in the first insult, contributing to an imbalance between factors that promote hepatic fat accumulation (free fatty acid flux to the liver and de novo lipogenesis) and factors that prevent fatty acid build-up (fatty acid export and oxidation). This renders hepatocytes susceptible to the secondary insults ('multiple hits') of adipokine-induced liver injury, oxidative and endoplasmic reticulum (ER) stresses, mitochondrial dysfunction, and hepatic apoptosis, which subsequently promote the transition from simple steatosis to NASH[22]. More recently, lipid partitioning in liver cells, as regulated by stearoyl-CoA desaturase-1 (SCD1), the enzyme that converts saturated free fatty acids (SFA) to monounsaturated free fatty acids (MUFA), and the ratio of SFA to MUFA, has been implicated in the progression from simple steatosis to NASH. A higher ratio has been suggested to confer a greater risk of hepatic cell damage by the influx of exogenous free fatty acids (FFA) and apoptosis, inflammation, and fibrosis[23].

In addition to hepatic insulin resistance, NAFLD is associated with a defect in insulin-mediated suppression of lipolysis, in keeping with insulin resistance in adipose tissues[24]. These findings suggest that insulin resistance might be an intrinsic defect in NAFLD, similar to that in type 2 diabetes, and that blunted insulin responsiveness at the level of the adipocytes might contribute to hepatic steatosis through excess free fatty acid flux to

the liver[25]. Isotope-tracer studies in obese humans with NAFLD on a low-fat diet showed that nearly 60% of hepatic triglycerides comes from FFA derived from adipose tissues, 26% from de novo lipogenesis and 15% from diet[26]. This would suggest that, in the absence of a high-fat diet, the increased release of fatty acids from adipose tissues is the predominant source of excess hepatic fat accumulation.

ROLE OF ADIPOKINES IN THE PATHOGENESIS OF NAFLD AND DIABETES

As obesity develops, changes in the size of adipocytes and fat deposits result in modifications of paracrine function in the adipose tissues leading to a chronic inflammatory state. In obese adipose tissues, the release of tumor necrosis factor-alpha (TNF-α) stimulates adipocytes to secrete monocyte chemoattractant protein-1 (MCP-1), leading to macrophage recruitment. Macrophage-related cytokine signaling promotes lipolysis through a decrease in lipid droplet stabilizing proteins (such as perilipin, fat specific protein 27). Lipolysis and the release of pro-inflammatory adipokines from adipose tissues; for example, leptin, further promote macrophage activation. The presence of activated macrophages, mediated by adipokine dysregulation, perpetuates a vicious cycle of macrophage recruitment, inflammatory cytokine production, lipolysis and impaired adipocyte function. This state of chronic inflammation stimulates nuclear factor-κB (NF-κB) and Jun N-terminal kinase (JNK) pathways in adipocytes[27]. We have shown that transgenic mice with selective inactivation of JNK in adipose tissues (*aP2-dn-JNK* mice) are protected against high fat diet (HFD)-induced obesity, insulin resistance and glucose intolerance. The expression of several pro-inflammatory cytokines, including TNF-α, interleukin-6 and MCP-1, are decreased in the transgenic mice, compared to wild-type littermates, whereas that of adiponectin, an anti-inflammatory adipokine, is increased. The messenger ribonucleic acid (mRNA) levels of hepatic gluconeogenic genes, phosphoenolpyruvate carboxykinase (*PEPCK*) and glucose 6-phosphatase (*G6Pase*), are also significantly decreased in *aP2-dn-JNK* mice, and the number of lipid-engorged hepatocytes is reduced, showing that inactivation of JNK attenuates HFD-induced hepatic steatosis and glucose production[28]. These findings suggest that interactions between inflammatory and metabolic pathways mediated by macrophages and adipocytes are important in the development of obesity-related insulin resistance, type 2 diabetes and NAFLD. In particular, the demonstration of a protective role in NAFLD of adiponectin, the most abundant adipokine in the circulation, and its reduction in patients with NAFLD[29] has generated extensive research into the role of adipokines in the pathogenesis of NAFLD and its complications.

Adiponectin

Adiponectin is an anti-inflammatory, insulin-sensitizing hormone secreted from adipocytes, and its circulating levels are inversely proportional to body mass index. Its expression is controlled by peroxisome proliferator-activated receptor-gamma (PPAR-γ), a transcription factor also predominantly expressed in adipose tissue[30]. Activation of PPAR-γ by its agonists, such as thiazolidinediones, increases adiponectin and reduces TNF-α expression[31]. Adiponectin circulates in the bloodstream as three oligomeric complexes: trimer, hexamer and high molecular weight (HMW) multimer, consisting of 18 or more monomers[32]. The gene that codes for human adiponectin is located on chromosome 3q27, a locus linked with the susceptibility to diabetes and obesity[33]. Another gene that is closely linked with NAFLD, the fetuin-A gene, also resides on chromosome 3q27; its expression is significantly elevated in mice with fatty liver and its plasma concentrations are raised in subjects with high liver fat[34]. High circulating fetuin-A levels are found in obesity[35] and confers increased risk of diabetes[36]. This lends further evidence of the interconnections between obesity, diabetes and NAFLD.

The protective effect of adiponectin on hepatic steatosis and liver injury, through its role in lipid homeostasis and anti-inflammatory action, has been shown in many experimental and clinical studies[29,37–39]. First, adiponectin expression from adipose tissues is markedly reduced in *ob*/*ob* mice (a leptin-deficient model with hyperinsulinemia, insulin resistance and steatosis). Recombinant adiponectin infusion into these obese mice alleviates steatosis, as shown by a significant reduction in hepatic fat content and serum alanine transferase levels[29] (Table 1). At a molecular level, the antisteatotic effect of adiponectin is mediated through the activation of 5-adenosine monophosphate-activated protein kinase (AMPK)[40]. AMPK activation phosphorylates acetyl coenzyme A carboxylase (ACC) and attenuates ACC activity, leading to enhanced fatty acid oxidation. Furthermore, AMPK activation downregulates the expression of sterol regulatory element-binding protein 1c (SREBP 1c), a key transcription factor for lipogenic genes, including ACC and fatty acid synthase (FAS), and glycerol-3-phophate acyltransferase (GPAT). Adiponectin administration has been shown to suppress the hepatic mRNA expression of ACC and FAS in alcohol-induced fatty liver disease in mice[29], and the expression of SREBP 1c in cultured hepatocytes and in the liver of +Lepr(db)/+Lepr(db) (*db*/*db*) mice[41]. In addition, adiponectin also stimulates peroxisome proliferator-activated receptor-alpha (PPAR-α), a transcription factor that controls genes encoding fatty acid oxidation enzymes[42]. In humans, serum adiponectin levels are negatively correlated with alanine aminotransferase (ALT) levels in obese Chinese individuals[29]. Serum total and HMW adiponectin levels have also been found to be lower in obese subjects with NAFLD compared with non-obese subjects without NAFLD, in association with increased insulin resistance and elevated hepatic SREBP 1c mRNA expression (real-time polymerase chain reaction)[43]. These animal and human studies have shown that adiponectin-mediated signalling leads to enhanced fatty acid oxidation and reduced lipid synthesis, thus preventing hepatic steatosis.

Second, adiponectin exerts an anti-inflammatory effect, thus protecting against secondary liver insults (in the 'multi-hit model'), largely by suppressing TNF-α function through

Table 1 | Adipokines in animal studies for non-alcoholic fatty liver disease and diabetes

Animal models/cell types	Adipokine	Effects of interventions	Reference
	Adiponectin	Treatment	
ob/ob mice	Reduced expression in adipose tissue	• Alleviates hepatic steatosis by reducing hepatic fat content and ALT levels • Reduces TNF-α production	29
db/db mice		• Suppresses hepatic SREBP-1 expression	41
db/db mice		• Alleviates hyperglycemia, hypertriglyceridemia, insulin resistance • Alleviates hepatic steatosis	32
	A-FABP		
Obese mice lacking A-FABP		• A-FABP deficiency protects against hepatic steatosis, insulin resistance, hyperinsulinemia and hyperglycemia; and reduces liver stearoyl-CoA desaturase-1, a rate-limiting enzyme that promotes hepatic fat accumulation	64,65
Diet-induced obese mice with NASH	Elevated hepatic expression in Kupffer cells	• A-FABP inhibition alleviates hepatic steatohepatitis	67
ob/ob mice		• A-FABP inhibition alleviates diabetes	75
	FGF21	Treatment	
Diet-induced obese mice		• Alleviates hepatic steatosis	83
Diet-induced obese mice		• Reduces triglyceride levels • Reverses fatty liver disease via the inhibition of SREBP-1	84
ob/ob mice *db/db* mice		• Reduces blood glucose and triglyceride levels	81

A-FABP, adipocyte-fatty acid binding protein; ALT, alanine transaminase; FGF21, fibroblast growth factor-21; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; SREBP-1, sterol regulatory element-binding protein; TNF-α, tumor necrosis factor-alpha.

inhibition of its expression and opposition to its actions[29,37,44]. Adiponectin treatment suppresses the augmented production of TNF-α in *ob/ob* mice[29]. In humans, decreased serum adiponectin levels and increased TNF-α and soluble TNF-α receptor 2 (TNFR2) levels correlate with the presence of NASH. Serum adiponectin levels are also inversely correlated with necro-inflammation in NASH[38]. However, the relationship between adiponectin and fibrosis is more controversial, with some authors reporting raised adiponectin levels found in cirrhosis[45], whereas others[46] have shown a negative correlation between adiponectin and advanced hepatic fibrosis. Supportive of the antifibrotic effect of adiponectin, obese and diabetic mice with increased fibrosis lack physiological upregulation in adiponectin levels[47].

Mitochondrial dysfunction contributes to the increased susceptibility to secondary liver injuries induced by obesity. Adiponectin has been shown to decrease hepatic mitochondrial dysfunction through induction of uncoupling protein 2 (UCP2), a mitochondrial inner membrane transporter. The protein and mRNA levels of UCP2 are decreased in liver tissues of adiponectin knockout mice and are upregulated by adiponectin treatment[44]. Adiponectin or UCP2 replenishment restores mitochondrial function and depletes lipid accumulation by reducing fatty acyl coenzyme A accumulation in livers of adiponectin knockout mice[48].

Hypoadiponectinemia is also implicated in the pathogenesis of type 2 diabetes in obese subjects and in individuals with impaired hepatic glucose production. The HMW oligomer of adiponectin has been shown to be the major active form responsible for its insulin-sensitizing effect in hepatocytes[49]. Similar to its antisteatotic effect, its glucose-lowering effect is also partly mediated through AMPK, which in turn inhibits hepatic glucose production by decreasing the expression of key gluconeogenic genes, such as phosphoenolpyruvate carboxykinase and G6Pase[49,50]. We have shown that the magnitude of AMPK phosphorylation in liver tissue and the metabolic effects of adiponectin in *db/db* mice correlate with the expression of HMW adiponectin oligomers[32]. Similar to its involvement in NAFLD, adiponectin deficiency is also implicated in mitochondrial dysfunction and glucose homeostasis in adipocytes[51]. It has been shown *in vitro* that both mRNA expression and secreted levels of adiponectin are decreased in adipocytes with mitochondrial dysfunction induced by oligomycin A, and the reduced levels of adiponectin and insulin sensitivity in mature adipocytes reflect a decrease in mitochondrial respiratory function[52].

As adiponectin plays such important causal roles in NAFLD and type 2 diabetes, linked by obesity-related insulin resistance, it has been recognised to be a potential biomarker for the detection and prediction of NAFLD and type 2 diabetes, or both. In NAFLD, a score combining serum adiponectin, homeostasis model assessment-insulin resistance (HOMA-IR) index (cut-off value ≥3.0) and serum type IV collagen 7S (cut-off value ≥5.0 ng/mL) predicted approximately 90% of patients with early-stage NASH, with a sensitivity of 94% and a specificity of 74%[53]. In another study, subjects with NASH had lower

Table 2 | Serum/hepatic adipokine levels in human subjects with non-alcoholic fatty lover disease and/or diabetes

Clinical conditions	Adipokine	Associated changes	Reference
	Adiponectin		
Obesity	Reduced	Negative correlation with ALT	29
Obesity and NAFLD	Reduced (HMW and total)	Negative correlation with insulin resistance	43
NASH	Reduced	Negative correlation with necro-inflammation	38
	Reduced		54
Early-stage NASH	Reduced		53
Advanced hepatic fibrosis	Reduced		46
Type 2 diabetes	Reduced (ratio of HMW to total adiponectin)		32
Type 2 diabetes	Reduced	Low baseline adiponectin and high TNF-α are predictive of diabetes	55,56 57
Type 2 diabetes and NASH	Reduced	Low adiponectin and transforming growth factor-β1 associated with advanced fibrosis in subjects with type 2 diabetes	59
	A-FABP		
NAFLD	Elevated hepatic expression	In liver biopsies of NAFLD subjects	69
NAFLD	Elevated	Positive correlation with TNF-α, HOMA-IR and metabolic syndrome	70
NAFLD	Elevated	Positive correlation with advanced grades of necro-inflammation and fibrosis	72
NAFLD and type 2 diabetes	Elevated		71
Type 2 diabetes	Elevated	Positive correlation with fasting glucose and 2-h glucose and predictor of T2DM	78
	FGF21		
NAFLD	Elevated FGF21 mRNA expression	In human liver tissues of NAFLD subjects Positive correlation with the degree of steatosis	85
IGT/type 2 diabetes	Elevated	Negative correlation with whole body insulin sensitivity Positive correlation with hepatic insulin resistance	88
Type 2 diabetes	Elevated	Independent predictor of type 2 diabetes	89
Insulin resistance	Elevated	Associated with diabetes and insulin resistance	90

A-FABP, adipocyte-fatty acid binding protein; ALT, alanine transaminase; FGF21, fibroblast growth factor-21; HMW, high molecular weight; HOMA-IR, homeostasis model assessment of insulin resistance; IGT, impaired glucose tolerance; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; TNF-α, tumor necrosis factor-alpha; TG, triglycerides.

adiponectin levels compared with healthy controls, and a formula incorporating adiponectin, leptin and ghrelin yielded an area under receiver operating characteristic of 0.789 ($P = 0.002$), sensitivity of 82% and specificity of 76% for NASH[54]. As for type 2 diabetes, a large prospective, case–control study has shown that mean adiponectin concentrations were significantly lower in individuals with incidental type 2 diabetes than in controls[55]. Low adiponectin levels at baseline was associated with an increased risk of diabetes in Caucasians[55,56]. Low adiponectin, together with high TNF-α at baseline, was also independently predictive of diabetes, and the combined use of serum adiponectin and TNFR2 levels were comparable to 2-h post-load glucose for diabetes prediction in Chinese subjects[57] (Table 2). In a recent study to characterize prediagnosis trajectories of adiponectin in individuals who developed type 2 diabetes, female subjects and those with early-onset diabetes (age at diagnosis <52 years) had a steeper decline in adiponectin levels than non-diabetic controls[58].

In patients with both type 2 diabetes and NAFLD, low adiponectin levels were independently associated with NASH in a cross-sectional study on type 2 diabetes patients with histologically-diagnosed NAFLD. Low adiponectin, together with transforming growth factor (TGF)-β1, were associated with advanced fibrosis, the more severe stage of NAFLD in subjects with type 2 diabetes. It has been postulated that type 2 diabetes patients with NAFLD might develop steatohepatitis and progressive fibrosis because of the lack of upregulation of adiponectin, which inhibits connective tissue growth factor (CTGF), a cell-adhesion factor for hepatic stellate cells and a deciding factor for the development of fibrosis[59]. CTGF has been described as a profibrotic factor that mediates some TGF-β1 responses, including apoptosis and fibrosis[59]. As type 2 diabetes patients have a more progressive course of NAFLD[60], these results suggest that hypoadiponectinemia, present in type 2 diabetes, might play a key role in the progression of NAFLD in type 2 diabetes patients.

Adipocyte Fatty Acid Binding Protein

Adipocyte fatty acid binding protein (A-FABP) is a cytosolic lipid-binding chaperone mainly expressed in mature adipocytes and activated macrophages. It was initially thought to be a solely intracellular protein, but our group has recently identified the circulating form of A-FABP in the human bloodstream[61]. It reversibly binds with a high affinity to hydrophobic ligands, such as saturated and unsaturated long-chain fatty acids, and functions as a fatty acid chaperone, which facilitates fatty acid signaling by targeting and transporting fatty acid metabolites to the lipid signal transduction pathway[62]. Its expression is highly regulated during differentiation of adipocytes, and transcription of its mRNA is controlled by fatty acids, insulin and PPAR-γ agonists[62]. Cross-sectional and longitudinal studies have reported positive associations between A-FABP levels and parameters of adiposity, insulin resistance and the metabolic syndrome[61,63].

In relation to NAFLD, mice lacking A-FABP were found to be strongly protected against hepatic steatosis[64] and had reduced liver SCD-1 activity, a rate-limiting enzyme important for the conversion of saturated to monounsaturated fatty acid that contributes to hepatic fat accumulation[65]. Hepatic expression of A-FABP in Kupffer cells has been shown to be elevated in chemically- and diet-induced obese mice with NASH, likely forming a feed-forward loop with JNK and c-Jun[66] (Table 1) to instigate an inflammatory response in Kupffer cells[67], the hepatic macrophages that are responsible for recruiting a cluster of pro-inflammatory cytokines to mediate transition from steatosis to steatohepatitis[68]. In keeping with this, elevated A-FABP expression has been observed in subjects with NAFLD[69] (Table 2). Cross-sectional studies have shown an association of elevated A-FABP levels with ultrasound-diagnosed NAFLD in both healthy[70] and type 2 diabetes subjects[71]. Furthermore, serum A-FABP levels can distinguish NASH from steatosis, and elevated A-FABP levels are independently associated with advanced grades of necro-inflammation and fibrosis in liver biopsies[72]. These results strongly support the role of A-FABP in the pathogenesis of obesity-related fatty liver disease.

As for its role in diabetes, mice lacking A-FABP are protected from development of insulin resistance, hyperinsulinemia, and hyperglycemia in the context of both dietary and genetic obesity[64,73]. Apolipoprotein E–/– mice lacking both adipocyte and macrophage fatty acid binding protein (FABP) have better insulin and glucose tolerance, and survival[74]. An orally active A-FABP inhibitor has been shown to be effective in alleviating diabetes in animal models, and obesity-induced adipose tissue JNK1 activity is attenuated in mice treated with A-FABP inhibitor[75].

In humans, significant reductions in A-FABP concentration, together with a decrease in TNFR2 and high sensitivity C-reactive protein, and an increase in adiponectin levels, were observed in obese individuals after bariatric surgery and intensive weight loss[76]. A-FABP contributes to an improvement in HOMA-IR index after weight loss, independent of pro-inflammatory/anti-inflammatory cytokine profile, thereby supporting its role in insulin-sensitivity pathways in the morbidly obese[76].

In a large population study, individuals with a genetic variant at the FABP gene locus, coinciding with the binding site for CCAAT/enhancer binding protein (C/EBP), had lower triglyceride levels and showed a reduced risk of obesity-induced type 2 diabetes. This particular mutation was found to alter C/EBP binding and reduce the transcriptional activity of the human FABP gene promoter, as well as the adipose tissue A-FABP expression of individuals carrying the variant[77]. The role of A-FABP in predicting diabetes has also been shown in a 10-year prospective study, whereby plasma A-FABP level correlated positively with fasting glucose and 2-h glucose and predicted the development of type 2 diabetes independent of the traditional risk factors that included obesity, insulin resistance, or glycemic indices[78].

Like adiponectin, A-FABP also has a dual role in the pathogenesis of NAFLD and type 2 diabetes, and would represent a useful biomarker for the prediction of NAFLD and type 2 diabetes. As animal studies have yielded promising results of A-FABP blockade in alleviating steatosis and impaired glucose tolerance[67,75], therapeutic inhibition of A-FABP can potentially target the triad of obesity, diabetes and fatty liver disease.

Fibroblast Growth Factor-21

Fibroblast growth factor 21 (FGF21), a polypeptide with 210 amino acid residues originally cloned from the mouse liver, is a metabolic hormone that regulates glucose and lipid metabolism. Obesity is associated with increased FGF21 expression in adipose tissues[79,80]. In obese rodents, adipocytes have been shown to be another important site of FGF21 production[80]. Thus, FGF21 can also be considered as an adipokine.

FGF21 activates cell signaling by binding to a heteromeric cell-surface receptor tyrosine kinase complex composed of β-Klotho and a fibroblast growth factor receptor, namely FGFR1c. Both β-Klotho and FGFR1c are abundantly expressed in white adipose tissue (WAT), where FGF21-regulated genes are involved in metabolic processes that include lipogenesis, lipolysis and fatty acid oxidation. Systemic administration and transgenic overexpression of FGF21 induce weight loss in obese mouse models through increases in energy expenditure without changing food intake[81].

Adipose FGF21 acts as an autocrine factor in the fed state by regulating the activity of PPAR-γ in adipose tissues. We have shown that both FGF21 mRNA expression and its protein release *in vitro* are markedly increased during conversion of human pre-adipocytes into mature adipocytes, showing a differentiation dependent expression of FGF21[80]. Chronic treatment of the PPAR-γ agonist, rosiglitazone, markedly enhances FGF21 production in both 3T3-L1 murine adipocytes and human adipocytes[80]. In obese mice, the degree of FGF21 expression in several types of adipose tissue has been shown to be markedly raised, to levels comparable to that of its expression in the liver[80].

In humans, serum FGF21 levels are also significantly elevated in obese subjects, thus providing evidence that adipose tissue is another important source of circulating FGF21[80]. *In vivo*, however, treatment with rosiglitazone leads to a reduction in circulating FGF21 levels in type 2 diabetes patients, likely as a result of the amelioration of diabetes-related metabolic dysfunction, such as insulin resistance and raised FFA levels.

In mice, FGF21 plays a physiological role in suppressing the rate of lipolysis, functioning as a metabolic regulator of lipid metabolism in concert with growth hormone[82]. Its role in alleviating hepatic steatosis has been shown by the effect of systemic administration of FGF21 in diet-induced obese mice (Table 1). Furthermore, adenovirus-mediated knockdown of hepatic FGF21 leads to the development of fatty liver and dyslipidemia as a result of the altered expression of several key genes involved in hepatic lipid metabolism[83]. Chronic treatment with recombinant FGF21 also reduces serum and hepatic triglyceride levels, and reverses fatty liver disease in diet-induced obese mice through the inhibition of SREBP-1, the key transcription factor for lipogenesis[84]. In human liver tissues, FGF21 mRNA expression increases with the degree of steatosis[85]. These findings might suggest a compensatory increase in hepatic FGF21 expression in response to FGF21 resistance, and that FGF21 resistance might have contributed to the pathogenesis of NAFLD. Alternatively, as in the case of type 2 diabetes, the increase in FGF21 levels might be secondary to the metabolic perturbations associated with insulin resistance. A recent study suggested that adipose tissue inflammation in obesity, involving the JNK1 pathway, can lead to the suppression of β-Klotho expression by TNF-α and hence impaired FGF21 action in adipocytes[86]. This might also explain the mechanism that leads to FGF21 resistance in NAFLD.

The role of FGF21 in glucose metabolism was first suggested by the finding of a high throughput screening that FGF21 was one of the agents capable of increasing glucose uptake in 3T3-L1 adipocytes[81]. The addition of recombinant FGF21 to adipocytes was found to induce insulin-independent glucose uptake by enhancing the expression of glucose transporter 1 (GLUT1). Subsequently, treatment with recombinant FGF21 was found to reduce blood glucose and triglycerides to near normal levels in both *ob*/*ob* mice and *db*/*db* diabetic mice[81] and chronic treatment with FGF21 in diabetic rhesus monkeys also ameliorated triglyceride and glucose controls[87].

Despite beneficial effects of FGF21 on glucose and lipid homeostasis in animal models, elevated circulating FGF21 levels are present in obese diabetic *db*/*db* mice and obese/overweight humans[80]. This elevation in FGF21 levels were also found in humans with IGT and type 2 diabetes, and correlated directly with hepatic insulin resistance and inversely with whole-body insulin sensitivity[88] (Table 2). A high FGF21 level in non-diabetic subjects has been shown to predict diabetes development during long-term follow up in the Hong Kong Cardiovascular Risk Factor Prevalence Study[89], suggesting that FGF21 resistance also occurs early in the course of dysglycemia and predisposes to diabetes development. The elevated serum FGF21 levels might be consequential to other metabolic disturbances, such as hyperinsulinemia or increased circulating FFA levels, in subjects with insulin resistance[90].

In summary, these findings show that FGF21, together with adiponectin and A-FABP, might serve as biomarkers for both NAFLD and dysglycemia.

THERAPEUTIC IMPLICATIONS

Metformin

Various antidiabetic agents have been shown to confer beneficial effects on NAFLD. Metformin has been shown to reduce insulin resistance and aminotransferase levels associated with NAFLD[91]. Like adiponectin, metformin exerts its insulin-sensitizing and antisteatotic effects, at least in part, through the AMPK-mediated pathway (Table 3). In adipose tissues, it has recently been shown that metformin improves insulin resistance by enhancing glucose transporter 4 (GLUT4) translocation through AMPK-mediated Cbl/c-Cbl-associated protein (CAP) signaling, thereby inhibiting differentiation of pre-adipocytes to adipocytes. Knockdown of AMPK and JNK blocks metformin-induced expression of CAP, implying that metformin stimulates the AMPK-JNK-CAP axis pathway[92]. Metformin also activates AMPK and reduces ACC protein levels in human adipose tissue[93]. In the liver, metformin acts through AMPK to stimulate fatty acid oxidation and decrease hepatic glucose production. Furthermore, metformin has been shown to induce hepatic FGF21 expression through AMPK activation. A strong dose-dependent increase in FGF21 expression was observed in both rat and human hepatocytes treated with metformin, an effect that was blocked by the addition of an AMPK-inhibitor[94]. Further studies are required to investigate if induction of hepatic FGF21 by metformin plays a significant role in mediating the metabolic benefits of metformin.

Thalizolidinediones

Pioglitazone, a PPAR-γ agonist, has been recommended to treat steatohepatitis in patients with biopsy-proven NASH[95]. Pioglitazone treatment in patients with NASH and dysglycemia (IGT or type 2 diabetes) was associated with improved aminotransferase levels, steatosis, inflammation and hepatocyte ballooning[96]. It exerts its therapeutic actions partly through adiponectin, with a 2.3-fold increase in plasma levels significantly associated with improved hepatic insulin sensitivity and histological improvement in hepatic steatosis, necro-inflammation and fibrosis *in vitro*[97]. Pioglitazone has also been shown to induce FGF21 expression in mouse and human adipocytes[98], and animal studies have shown its role as an autocrine factor regulating the activity of PPAR-γ in adipose tissues[99]. Whether FGF21 is involved in the increase in adiponectin expression by PPAR-γ and hence the protection against NASH remains speculative.

Glucagon-Like Peptide-1 Agonists and Enhancers

Glucagon-like peptide-1 (GLP-1) suppresses hepatic lipogenesis through activation of the AMPK pathway in hepatocytes. The

Table 3 | Mechanisms of action of current antidiabetic agents on non-alcoholic fatty liver disease

Class of antidiabetic agent	Example	Primary mechanism	Effects on liver or adipose tissue hormone expression	Actions in NAFLD	Reference
Biguanides	Metformin	Activates AMPK	Induces FGF21 expression in hepatocytes	Improves insulin resistance Reduces aminotransferase levels Reduces hepatic glucose production Stimulates fatty acid oxidation in liver	91,92,94
Thiazolidinediones	Pioglitazone	Activates nuclear transcription factor PPAR-γ	Increases circulating adiponectin level Induces FGF21 expression in adipocytes	Reduces aminotransferase levels Reduces hepatic steatosis, inflammation and fibrosis Improves hepatic insulin sensitivity	96–98
DPP-4 Inhibitors	Sitagliptin, vildaglitin, linagliptin, saxagliptin	Inhibits DPP-4 activity, increasing postprandial GLP-1 concentrations		Improves liver enzyme levels and hepatocyte ballooning Reduces plasma glucose and liver enzyme levels	102,103
GLP-1 Receptor Agonists	Exenatide, liraglutide	Activates AMPK in hepatocytes	Increases hepatic FGF21 expression and plasma FGF21 level	Reduces hepatic lipogenesis Reduces diet-induced hepatic pro-inflammatory response Improves insulin sensitivity	100,101

AMPK, adenosine monophosphate-activated protein kinase; CAP, Cbl/c-Cbl-associated protein; DPP-4, dipeptidyl peptidase-4; FGF-21, fibroblast growth factor-21; GLP-1, Glucagon-like peptide-1; GLUT-4, glucose transporter 4; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PPAR-γ, peroxisome proliferator-activated receptor-gamma.

inhibitory effects of GLP-1 on hepatic fat accumulation and diet-induced hepatic pro-inflammatory response suggest a therapeutic role of GLP-1 agonists in NAFLD[100].

Liraglutide, a long-acting GLP-1 agonist, increases FGF-21, FGFR mRNA and protein expression, and improved insulin sensitivity, in a mouse model of insulin resistance induced by a combination of adiponectin and apolipoprotein E deficiency, and high fat[101]. In addition, preliminary evidence suggests that dipeptidyl peptidase IV (DPP-4) inhibitors, which enhances endogenous GLP-1 levels by inhibiting its rapid degradation by DPP-4, ameliorate liver enzymes and hepatocyte ballooning in NASH patients with type 2 diabetes[102]. In a pilot study, significant reduction in plasma glucose, hemoglobin A1c (HbA_{1c}) and liver enzyme levels were observed after 4 months of treatment with sitagliptin in NAFLD patients with type 2 diabetes[103]. More studies are required to determine the role of DDP-4 inhibitors on adipokines, which may or may not be similar to that of GLP-1 agonists.

Future Therapeutic Targets

Agents that enhance adiponectin production might represent potential targets for the treatment or prevention of NAFLD and diabetes. Such agents might be derived from natural products, as exampled by the identification of two naturally-occurring compounds (astragaloside II and isoastragaloside I) from the widely used medicinal herb, Radix Astragali, which can selectively increase adiponectin secretion in primary adipocytes. The two compounds further enhance adiponectin production in addition to the effect of rosiglitazone. These changes are associated with an alleviation of hyperglycemia, glucose intolerance and insulin resistance, and might also provide beneficial effects for NAFLD[104]. Recently, A-FABP blockade has also shown promising results in animal models in alleviating obesity-related NAFLD[62,67]. Therefore, therapeutic targets based on selective A-FABP inhibition are also a promising area for further investigation. Whether FGF21, which is being actively researched in preclinical studies for the treatment of diabetes, can ameliorate NAFLD in humans remains to be investigated.

ROLE OF ADIPOKINES IN THE SCREENING FOR DYSGLYCEMIA IN NAFLD

Importance of Screening

NAFLD renders a person 1.6-times more likely to develop diabetes[105]. Obesity also increases the risk of diabetes in people with NAFLD, as the incidence in an urban Chinese population was shown to be highest in obese subjects with NAFLD (23.2%), when compared with the non-obese group with NAFLD (11.1%) and those without NAFLD (4.3%)[16]. In addition, diabetes mellitus, obesity and old age were significant predictors of severe liver fibrosis[60]. Mortality amongst community-diagnosed NAFLD patients was higher than the general population, and was associated with impaired fasting glucose, old age and cirrhosis[10]. Screening for dysglycemia in NAFLD should include an oral glucose tolerance test (OGTT) to diagnose prediabetes (impaired fasting glucose [IFG] and IGT), as individuals with prediabetes are already at risk of developing diabetes-related complications[106]. Furthermore, NAFLD patients with prediabetes had worse hepatic insulin resistance than NAFLD patients with normal glucose tolerance and those without NAFLD[107]. Indeed, NAFLD patients with prediabetes had a similar degree of muscle and liver insulin resistance as NAFLD patients with type 2 diabetes[107].

Oral Glucose Tolerance Test for Diagnosis of Dysglycemia in NAFLD

To detect prediabetes and type 2 diabetes, 75-g OGTT, rather than fasting glucose alone, has been recommended in NAFLD patients, as fasting glucose (≥7.0 mmol/L) has been found to considerably underestimate the diabetes prevalence in Hong Kong Chinese[108], and IGT with normal fasting plasma glucose is common (47%) among Hong Kong Chinese with biopsy-proven NAFLD[109]. However, OGTT is notorious for being cumbersome to carry out, and has poor reproducibility with large intra-individual variation in glucose responses[110]. The alternative use of HbA_{1c} is also limited by its lower sensitivity in identifying prediabetes and type 2 diabetes than OGTT[111]. The measurement of adipokines could potentially serve to provide biomarkers that can enhance the detection of dysglycemia in NAFLD without the use of OGTT.

Role of Adipokines in Detecting Dysglycemia in NAFLD

We have discussed the potential diagnostic and prognostic roles of adipokines in detecting diabetes, as well as their effects in currently available antidiabetic agents in the treatment of NAFLD. In essence, low adiponectin, together with high TNF-α at baseline, is independently predictive of diabetes[57], with a performance comparable to that of 2-h plasma glucose post OGTT. High A-FABP and FGF21 levels are also a strong predictor of diabetes[78,89]. In the context of NAFLD, low adiponectin levels were independently associated with NASH in type 2 diabetes patients with NAFLD[59]. Further studies will be required to evaluate the diagnostic roles of adipokines specifically in patients with NAFLD.

CONCLUSION

Adipose tissue dysfunction is characterized by inflammation and adipokine dysregulation, and subsequent ectopic fat deposition in the abdominal viscera and liver, and insulin resistance. It significantly contributes to the development of obesity-related conditions, including NAFLD and diabetes mellitus. Adipokines are important mediators of both lipid and glucose homeostasis. Adiponectin has antisteatotic, anti-inflammatory and insulin-sensitizing properties by promoting free fatty acid oxidation, reducing fatty acid influx to liver and de novo lipogenesis, as well as suppressing the action of pro-inflammatory cytokines and gluconeogenesis. A-FABP facilitates fatty acid signaling, which promotes hepatic fat accumulation and inhibition of A-FABP in mice, has been shown to alleviate NAFLD and diabetes. Recombinant FGF21 administration has been shown to reverse fatty liver disease and improve glucose control in animal models. These adipokines have been implicated in currently-available antidiabetic agents with beneficial effects on NAFLD. Adipokine-based therapeutic agents for NAFLD and diabetes would represent a promising area for further investigation.

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