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Review

Bidirectional relationships and disconnects between NAFLD and features of the Metabolic Syndrome

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Abstract: Non-alcoholic fatty liver disease (NAFLD) represents a wide spectrum of liver disease from simple steatosis, to steatohepatitis, (both with and without liver fibrosis), cirrhosis and end-stage liver failure. NAFLD also increases the risk of hepatocellular carcinoma (HCC) and both HCC and end stage liver disease may markedly increase risk of liver-related mortality. NAFLD is increasing in prevalence and is presently the second most frequent indication for liver transplantation. As NAFLD is frequently associated with insulin resistance, central obesity, dyslipidaemia, hypertension and hyperglycaemia, NAFLD is often considered the hepatic manifestation of the metabolic syndrome. There is growing evidence that this relationship between NAFLD and metabolic syndrome is bidirectional, in that NAFLD can predispose to metabolic syndrome features, which can in turn exacerbate NAFLD or increase the risk of its development in those without a pre-existing diagnosis. Although the relationship between NAFLD and metabolic syndrome is frequently bidirectional, recently there has been much interest in genotype / phenotype relationships where there is a disconnect between the liver disease and metabolic syndrome features. Such potential examples of genotypes that are associated with a dissociation between liver disease and metabolic syndrome are patatin-like phospholipase domain-containing protein-3 (PNPLA3) (I148M) and transmembrane 6 superfamily member 2 protein (TM6SF2) (E167K) genotypes. This review will explore the bidirectional relationship between metabolic syndrome and NAFLD, and will also discuss recent insights from studies of PNPLA3 and TM6SF2 genotypes that may give insight into how and why metabolic syndrome features and liver disease are linked in NAFLD.

Keywords: NAFLD; metabolic syndrome; insulin resistance; PNPLA3

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a considerable public health concern, and is the commonest cause for chronic liver disease in the developed world [1,2]. Worldwide prevalence of NAFLD is estimated to be in the region of 20% in the general population [3]. NAFLD represents a disease spectrum ranging from hepatic steatosis, to non-alcoholic steatohepatitis, to cirrhosis, end-stage liver failure and hepatocellular carcinoma. The accepted definition of NAFLD is a hepatic triglyceride content of greater than 5.5%, as determined from analysis of the Dallas Heart Study cohort [4]. The metabolic syndrome is a collection of underlying risk factors for cardiovascular disease with an estimated prevalence in the USA of 34% [5].

The relationship between NAFLD, obesity, insulin resistance and type 2 diabetes is a complex one. NAFLD has traditionally been considered to be the hepatic manifestation of the metabolic

syndrome, due to the close association between NAFLD and the various component features of the metabolic syndrome such as abdominal obesity, hypertension, elevated fasting plasma glucose, raised serum triglycerides and low high-density lipoprotein cholesterol (HDL-C) concentrations. Many epidemiological studies have demonstrated an association between NAFLD and the metabolic syndrome [6-8].

There is now a growing body of evidence supporting the idea that there is a bidirectional relationship between NAFLD and features of the metabolic syndrome, with insulin resistance being the central pathophysiological process common to both conditions. As such there currently exists and "chicken and egg" debate in the literature regarding the temporal relationship between NAFLD and the metabolic syndrome, with no clear consensus about which is considered to generally occur first. A recent study has demonstrated a reciprocal causality between NAFLD and metabolic syndrome in a Chinese population, with metabolic syndrome being found to have a greater effect on incident NAFLD in terms of causality than NAFLD does on incident metabolic syndrome [9].

In addition to this there are recognised situations whereby there is an apparent disconnect between NAFLD and insulin resistance / metabolic syndrome features, and these generally arise as a result of particular genetic polymorphisms such as in the patatin-like phospholipase domain-containing protein-3 (PNPLA3) gene.

This review will attempt to review the available evidence regarding the bidirectional relationship between NAFLD and components of the metabolic syndrome, as well as to explore the potential disconnects that may exist between the two due to genetic variability and inherited metabolic disease.

Association between NAFLD and components of the metabolic syndrome

There have been various diagnostic criteria available for the diagnosis of metabolic syndrome, and these have changed subtly over recent years. The most commonly used criteria are those published by the International Diabetes Federation in 2009. It should be noted that these most recent criteria advocate using population- and country- specific definitions for abdominal obesity [10]. **Table 1** outlines the various diagnostic criteria available.

72 **T**able 1 – Diagnostic criteria available for metabolic syndrome

	WHO (1999)	**NCEP (2001)**	**IDF (2005)**	**IDF (2009)**
Required	Insulin resistance	Nil	Waist circumference ≥94cm in men, ≥80cm in women	Nil
Number of features	≥ 2 of:	≥ 3 of:	≥ 2 of:	≥ 3 of:
Obesity	Waist/hip ratio of > 0.9 in men, >0.85 in women or BMI ≥30	Waist circumference ≥102cm in men, ≥88cm in women		Waist circumference – population specific definitions
Triglycerides	≥ 150mg/dL (1.7 mmol/L)	≥ 150mg/dL (1.7 mmol/L)	≥ 150mg/dL (1.7mmol/L)	≥ 150mg/dL (1.7mmol/L)
HDL-cholesterol	<40mg/dL (1mmol/L) in men, <50mg/dL (1.3 mmol/L) in women	<40mg/dL (1mmol/L) in men, <50mg/dL (1.3 mmol/L) in women	<40mg/dL (1mmol/L) in men, <50mg/dL (1.3mmol/L) in women	<40mg/dL (1mmol/L) in men, <50mg/dL (1.3mmol/L) in women
Hypertension	≥ 140/90 mmHg	≥ 135/85 mmHg	≥ 135/85 mmHg	≥ 135/85 mmHg
Glucose		110 mg/dL (6.1 mmol/L)	100 mg/dL (5.6 mmol/L)	100 mg/dL (5.6 mmol/L)
Microalbuminuria	Albumin/creatinine ratio > 30 mg/g; albumin excretion rate > 20 mcg/min			

73 WHO, World Health Organisation; NCEP, National Cholesterol Education Program; IDF, international diabetes federation; DM, diabetes mellitus.

NAFLD can occur in individuals who are not obese [11,12], however this is more unusual and generally NAFLD is closely related to increased central adiposity. NAFLD is commonly associated with all of the component features of the metabolic syndrome, and nearly two thirds of people with obesity and type 2 diabetes demonstrate hepatic steatosis [13,14]. One study identified hepatic steatosis via ultrasonography in 50% of patients with hyperlipidaemia [15]. NAFLD is also associated with arterial hypertension and cross-sectional studies have demonstrated that approximately 50% of people with essential hypertension also have NAFLD [16,17]. Importantly, in those people with NAFLD the presence of multiple features of the metabolic syndrome is associated with more severe liver disease and a higher likelihood of progression to NASH and cirrhosis [18,19].

NAFLD as a risk factor for and precursor to the metabolic syndrome

There is evidence to suggest that NAFLD, rather than being simply the hepatic manifestation of the metabolic syndrome, may in fact be a necessary first step in its development.

When the link between NAFLD and insulin resistance was initially described by Day et al, it was proposed as part of a "two hit hypothesis" [20]. Here, the "first hit" was increased triglyceride accumulation as a result of insulin resistance and increased delivery of free fatty acids to the liver, followed by a "second hit" of hepatic oxidative stress resulting in increased lipid peroxidation. This was said to then lead inexorably to hepatocyte injury, inflammation and fibrosis, with the potential for progressive liver damage. It has subsequently been suggested that pathogenesis of NAFLD may in fact reflect "multiple parallel hits" which all contribute to an environment of hepatic inflammation with the involvement of cytokines and adipokines from extrahepatic tissues such as the gut and adipose tissue [21].

From a basic science perspective, there is reason to believe that hepatic lipid accumulation could be a cause and a perpetuating factor for the development of insulin resistance. There is currently much interest in fully elucidating the role that protein kinase C-ε (PKC- ε) may play in this relationship. An elegant study conducted by Samuel et al investigated PKC- ε and how it may link NAFLD and insulin resistance [22]. They observed that rats that were fed a 3 day high-fat diet developed marked hepatic steatosis and hepatic insulin resistance as determined by hyperinsulinaemic-euglycaemic clamp studies. Here, PKC- ε was activated but other forms of PKC were not. Crucially, the authors then went on to attenuate the expression of PKC- ε using an anti-sense oligonucelotide directed at PKC- ε and they noted that this protected the rats from steatosis-induced hepatic insulin resistance and also reversed defects that they had observed in insulin receptor signalling function. It should be noted that both hepatic diacylglycerol and triacylglycerol content were not affected by this intervention suggesting that the hepatic lipid accumulation is a prerequisite for insulin resistance. This relationship has also been investigated in humans, in a study of 37 obese non-diabetic individuals awaiting bariatric surgery [23]. Here it was observed that hepatic diacylglycerol content from liver biopsy specimens was the strongest predictor of insulin resistance and accounted for 64% of the variability in insulin sensitivity. Hepatic diacylglycerol content was strongly correlated with activation of PKC- ε. Given this evidence, a model has emerged whereby increases in liver diacylglycerol content result in activation of PKC- ε, translocation of PKC- ε in the cell membrane, inhibition of hepatic insulin signalling and the resulting generation and maintenance of hepatocyte insulin resistance.

More recently there has been interest in the hepatokine, fetuin B. This compound has been shown to be increased in obese rodents [24]. It has also been shown that overnutrition in experimental mice results in hepatic steatosis, and this alters the hepatocyte protein secretion profile leading to increased secretion of fetuin B [25]. The authors of this study went on to further study the effects of fetuin B in vivo and observed that injecting recombinant fetuin B intraperitoneally into mice significantly impaired glucose tolerance when compared with controls. In addition to this, silencing fetuin B gene expression using short hairpin RNA was found to increase glucose tolerance. As such, fetuin B provides an example of how hepatic steatosis can be linked to the development of

insulin resistance and thus the metabolic syndrome. Other hepatokines such as FGF21 and selenoprotein P are thought to be play a role in the pathophysiology of insulin resistance with action on the liver and other tissues, however it is less clear how they fit into the relationship between hepatic steatosis and the metabolic syndrome.

It is known that most people with NAFLD also have insulin resistance, however most do not exhibit all of the features of the metabolic syndrome [26]. This could indicate that hepatic steatosis is required as a prerequisite for the development of further metabolic disease such as altered glucose and lipid metabolism. There is now a significant body of clinical evidence for NAFLD preceding, and being a strong risk factor for, development of the metabolic syndrome and its various components. A large prospective cohort study looked at 17,920 individuals from a Han Chinese population and followed them up over a 6 year period [27]. These individuals did not have metabolic syndrome at baseline, and the authors identified NAFLD as an independent risk factor for its development with an adjusted hazard ratio of 1.55 (95% confidence intervals 1.39-1.72). This observation of NAFLD as an independent risk factor for the development of the metabolic syndrome has also been made in a variety of other populations such as North American [28], western Australian [29], Korean [30], Japanese [31] and south Indian [32].

A large prospective cohort study of over 22,000 Korean men demonstrated that NAFLD is an independent risk factor for incident arterial hypertension, and that risk increases with severity of NAFLD [33]. This study replicated the findings of an earlier, smaller prospective study which demonstrated that NAFLD was an independent risk factor for the development of prehypertension [34]. Another prospective cohort study examined 1521 people and stratified them on the basis of their fatty liver index score (a surrogate marker of hepatic steatosis) [35]. It was observed that NAFLD, as diagnosed using fatty liver index score, was an independent risk factor for incident arterial hypertension. Finally, a retrospective cohort study of 11,448 individuals without hypertension revealed that the development of incident fatty liver disease over a five year period was associated with increased risk of incident hypertension [36].

A retrospective study of a Korean occupational cohort of 13,218 individuals observed that development of new fatty liver was associated with incident diabetes [37]. There are many prospective studies in the literature that demonstrate that NAFLD, and the surrogate markers with which it is associated, is a key risk factor and precursor for the development of type 2 diabetes [29,38-46]. **Table 2** summarises the characteristics of these key studies.

155 Table 2 – Characteristics of prospective studies linking hepatic steatosis to the development of type 2 diabetes

156

Study	Country / population	Sample size	NAFLD diagnostic method / surrogate marker used	Duration of follow-up	Key findings	Limitations of study
Vozarova 2002	Pima Indians aged 18-50	173 women, 278 men	ALT, AST and GGT concentrations	6.9 years average	High baseline ALT associated with increased risk of incident DM	Only surrogate markers used, no control for alcohol / hep C
Lee 2003	Korean men aged 25-55	4088 men	GGT concentration	4 years	Strong relationship between baseline GGT and risk of incident DM	Only studied men, only used surrogate marker
Hanley 2005	USA non-Hispanic whites and African American adults	910 women, 715 men	ALT, AST and ALP concentrations	5.2 years average	ALT and ALP in upper quartile at baseline significantly increased risk of metabolic syndrome	Only surrogate markers used for NAFLD diagnosis
Ekstedt 2006	Swedish NAFLD patients	87 men, 42 women	Biopsy-proven NAFLD	13.7 years average	Marked increase in proportion of patients with DM over period of study	No control group, no baseline glycaemic data to compare
Monami 2008	Florence aged 40-75	3124 total	ALT, AST and GGT concentrations	40 months average	Baseline GGT near upper limit of normal predicts incident DM	Study population participated in screening programme for diabetes, may not be representative
Goessling 2008	New England adults, all white	1575 women, 1237 men	ALT and AST concentrations	20 years	Increased ALT associated with higher risk of DM and metabolic syndrome, increased AST associated with incident DM risk	Homogenous study population, only surrogate markers used
Adams 2009	Western Australian adults	115 women, 243 men	NAFLD diagnosed with ALT after exclusion of other causes	11 years	NAFLD associated with higher risk of incident diabetes	Not an independent predictor if adjusted for WC, hypertension or insulin resistance
Ryu 2010	Korean men aged 30-65	9148 men	GGT concentrations	4.1 years average	Increase in GGT during study period predicted incident metabolic syndrome	Did not use accepted criteria for diagnosis of metabolic syndrome
Balkau 2010	Western France, aged 30-65	1950 women, 1861 men	NAFLD diagnosed using fatty liver index (FLI) score	9 years	Higher FLI score at baseline predicted incident DM	Used FLI rather than formal diagnostic methods
Sung 2011	Korean adults	7236 men, 3855 women	NAFLD diagnosed with ultrasound scan	5 years	Presence of fatty liver on ultrasound strongly predicted incident DM	Ultrasound relatively insensitive for diagnosis

Of particular interest is a longitudinal cohort study in which the authors followed up 358 individuals (109 with NAFLD, 249 without NAFLD) over an 11 year period [29]. After excluding those who had type 2 diabetes at baseline, they observed that those with NAFLD were significantly more likely to develop diabetes during the follow up period than those without. Similarly, they observed the same regarding who would go on to develop the metabolic syndrome. Also, a retrospective study of a Korean occupational cohort of 12,853 individuals demonstrated that the clustering of insulin resistance, overweight / obesity and hepatic steatosis markedly increased risk of incident type 2 diabetes [47]. The fully adjusted odds ratio for those with all 3 factors and risk of incident diabetes at 5 years follow-up was 14.13 (95% confidence intervals 8.99-22.21).

In addition to this, a meta-analysis has been performed recently which concluded that the presence of NAFLD doubles an individual's risk of developing type 2 diabetes in later life [48]. It would seem that there may be subsets of patients with NAFLD that have different levels of risk of type 2 diabetes, with one small study suggesting that the presence of biopsy-proven NASH is a greater risk factor than steatosis alone [41]. This is consistent with the accepted notion that individuals with nonalcoholic steatohepatitis (NASH) will tend to have a greater burden of metabolic disease.

Metabolic syndrome as an initiating or aggravating factor for liver disease

In addition to the evidence from the literature that NAFLD may predispose individuals to developing or worsening insulin resistance and the metabolic syndrome, there is also growing evidence that insulin resistance may contribute to progressive liver damage.

Of particular interest is the role played by plasminogen activator inhibitor 1 [49]. PAI-1 is a member of the serine protease inhibitor family, and acts as a key mediator in the fibrinolytic system. In tissues with a significant degree of fibrosis, concentrations of PAI-1 are elevated leading to an inhibition of tissue proteolytic activities, a decreased rate of collagen degradation and increased tissue fibrogenesis [49]. Increased PAI-1 levels are associated with obesity, insulin resistance, type 2 diabetes and dyslipidaemia [50,51]. Specifically it has been shown that PAI-1 concentrations measured in subcutaneous adipose tissue biopsy samples from individuals with nascent metabolic syndrome are significantly higher than those in control samples [52]. It has also been observed in a human hepatocyte cell line that tumour necrosis factor α (TNF- α) is able to induce the expression of PAI-1, leading to increased hepatic fibrosis and atherosclerosis in insulin-resistant individuals [53]. There is also a wealth of evidence in the literature regarding the role of PAI-1 in initiating and perpetuating hepatic fibrosis [49]. As such this provides evidence of a causative role for insulin resistance and obesity in the generation of ongoing hepatic fibrosis.

In addition to this, there is evidence that other inflammatory cytokines originating from white adipose tissue as a result of obesity and insulin resistance may play a significant role in hepatic fibrosis and inflammation. It has been known for some time that white adipose tissue is not metabolically inert but is a complex organ that can become active in the obese, insulin-resistant state leading to the production of various pro-inflammatory cytokines [54-55]. These cytokines include interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-18 (IL-18), complement component 3 (C3), TNF-α, PAI-1, adiponectin, leptin, resistin, apelin, vaspin and visfatin. There is evidence that these inflammatory mediators could play a role in the progression of liver disease from "simple" steatosis to NASH [56,57], and also that they may stimulate the differentiation of stellate cells in the liver into myofibroblast-like cells resulting in a more fibrogenic environment [58]. IL-1β, IL-6 and TNF-α are traditionally considered to be pro-inflammatory cytokines, and are all thought to play a role in the pathogenesis of NASH and its associated fibrosis [59,60]. More recently it has been suggested that the balance of pro- and anti-inflammatory mediators can lead to alterations in the gut microbiota and that this may have a significant impact on the progression of hepatic steatosis to NASH [61]. It has also been suggested that apoptosis of hepatocytes could be an important factor in liver damage and specifically progression to NASH [62,63]. Recent findings indicate that patients with a higher degree of insulin resistance exhibit

greater evidence for apoptosis of hepatocytes in liver biopsy specimens of morbidly obese individuals, and it has been speculated that this may be mediated by inflammatory cytokines [64]. These studies all provide evidence for a causative link between insulin resistance and hepatic damage mediated in part by inflamed, endocrinologically-active adipose tissue.

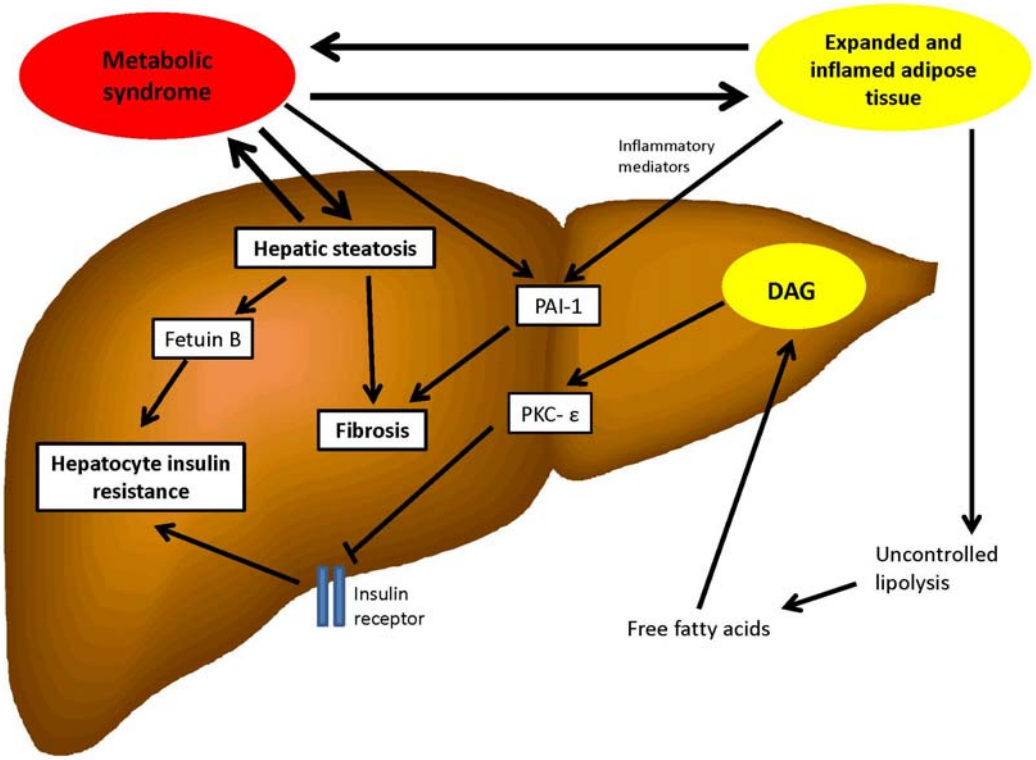
There is also clinical evidence that insulin resistance and the metabolic syndrome can cause a worsening of liver disease. A retrospective study of 103 individuals with NAFLD examined histological findings from paired liver biopsy specimens with an average interval of 3 years [65]. The authors observed marked variability in the progression of histological features of NAFLD between the 2 time points, but noted that those individuals with diabetes were at higher risk than non-diabetic people for progression of fibrosis. It is also established in the literature that metabolic syndrome and type 2 diabetes are strongly associated with severe liver disease such as cirrhosis and hepatocellular carcinoma [66-69]. It appears from the literature that individuals with type 2 diabetes and NAFLD combined are at markedly greater risk of more severe liver disease than those with NAFLD alone, and their liver-related mortality is greater.

There are a variety of cross-sectional studies available that demonstrate that metabolic syndrome and its components are associated with an increased risk of NAFLD in a variety of populations including North American [70], Mexican [71], Taiwanese [72] and Japanese [26]. However, given the cross-sectional nature of these studies they do not provide real evidence of a causative link. Of interest is a recent longitudinal prospective cohort study of 15,791 Han Chinese individuals followed up over a 6 year period [73]. They observed 3913 new cases of NAFLD in this population, and risk of incident NAFLD was markedly higher in those with metabolic syndrome. After adjusting for possible confounding factors such as age, diet, sex, smoking status and level of physical activity, the hazard ratio for incident NAFLD was found to be 1.94 (95% confidence intervals 1.78-2.13). The authors also observed that hazard ratios for incident NAFLD increased the more components of the metabolic syndrome were present at baseline, reaching 3.51 (95% confidence intervals 3.15-3.91) when 3 components were present as compared with individuals who exhibited no metabolic syndrome components. **Figure 1** summarises the bidirectional relationship between hepatic steatosis and the metabolic syndrome with regards to the various aspects described above.

Evidence for a Disconnection Between Hepatic Steatosis and Metabolic Syndrome

Despite the clear bidirectional causal links between NAFLD and the metabolic syndrome, there are certain situations where this appears to not be the case. In such scenarios there is a clinical disconnect between NAFLD and insulin resistance. Several groups have demonstrated that it is possible experimentally to induce either insulin resistance or hepatic steatosis individually without the presence of the other. The first evidence that hepatic steatosis could occur independently of insulin resistance was published in 2007 [74]. Here mice were raised which over-expressed acyl-CoA:diacylglycerol acyltransferase 2 (DGAT 2), an enzyme which acts to catalyze the final step of hepatic triglyceride biosynthesis. These mice were observed to develop marked hepatic steatosis in the absence of any abnormalities in plasma glucose and insulin levels, glucose and insulin tolerance, or infusion rates during hyperinsulinaemic euglycaemic clamp experiments. A subsequent study investigated variability in the DGAT2 gene to see if this relationship could also be found in humans. The authors investigated 187 individuals from south Germany, and observed 2 single nucleotide polymorphisms (SNPs) in DGAT2 that were associated with smaller decreases in liver fat following an exercise programme than wild type genotype [75]. There were no observed changes in insulin sensitivity among the different genotypes and thus the authors concluded that DGAT2 may play a role in mediating a disconnection between insulin resistance and hepatic steatosis. Additionally, it has been observed that inhibiting secretion of very low density lipoprotein (VLDL) from the liver by a genetic modification or diet-induced choline deficiency in a mouse model results in accumulation of hepatic triglyceride without causing insulin resistance [76,77].

Figure 1 – Schematic demonstrating the bidirectional interactions between hepatic steatosis and metabolic syndrome and aspects of how these are mediated. DAG; diacylglycerols, PKC- ε; protein kinase C ε, PAI-1; plasminogen activator inhibitor-1.

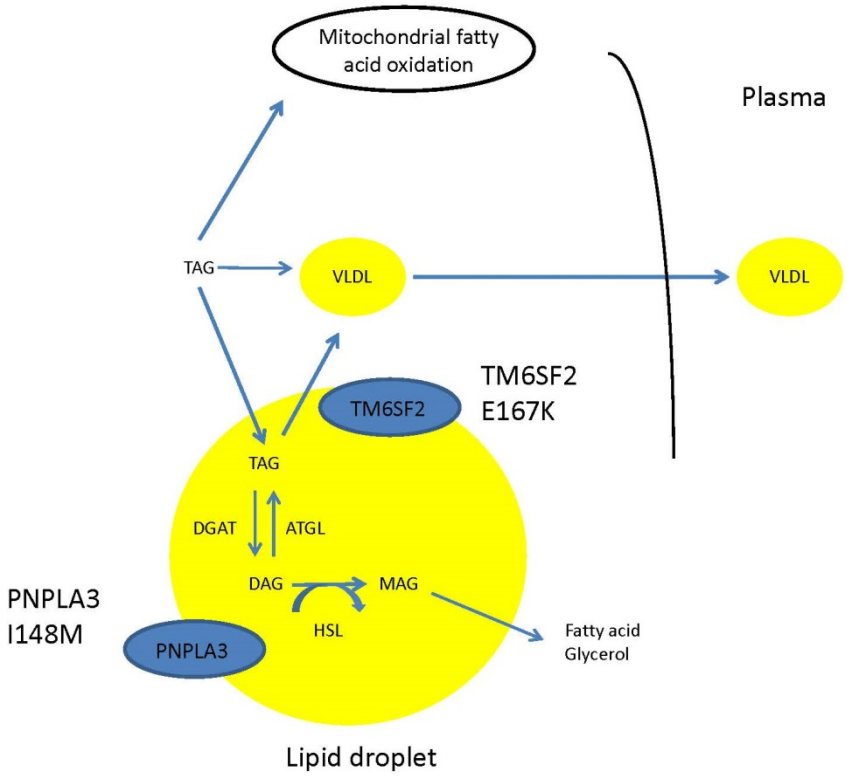


More recently, there has been much interest focused on the patatin-like phospholipase domain-containing protein-3 (PNPLA3) gene, which encodes for a protein called adiponutrin. The exact role of this adiponutrin is currently unclear, however it is recognised as being a membrane-associated protein expressed in hepatic and adipose tissue that possesses lipogenic and lipolytic activities. There is evidence to suggest that it is located in lipid droplets and may play a role in triglyceride hydrolysis [78]. PNPLA3 gene expression is upregulated following the post-prandial insulin spike, and downregulated following fasting. It was reported in 2008 that a particular allele in PNPLA3 (I148M or rs738409) was strongly associated with increased hepatic steatosis and hepatic inflammation, with individuals homozygous for I148M exhibiting twice the level of hepatic fat content than non-carriers [79]. Interestingly, it was also observed that I148M carrier frequency was highest in Hispanic populations who are thought to have highest susceptibility to NAFLD, and regression analysis demonstrated that the presence or absence of this PNPLA3 variant along with another (453I) accounted for 72% of the observed ethnic differences in levels of hepatic steatosis from the Dallas Heart Study. It was subsequently reported that the I148M variant has a marked effect on enzyme activity and results in a disruption to normal hydrolysis of triglycerides leading to impaired secretion of very low density lipoproteins (VLDL) [80,81]. Interestingly, it has subsequently been demonstrated that the association between the I148M variant and NAFLD in independent of insulin sensitivity as measured by hyperinsulinaemic euglycaemic clamp, as well as central obesity [82,83]. Therefore the PNPLA3 I148M variant provides an example of how hepatic steatosis can occur in humans independently of insulin resistance and the metabolic syndrome.

A similar scenario has been identified more recently with the transmembrane 6 superfamily member 2 (TM6SF2) gene. TM6SF2 is expressed largely in the liver and intestine and is thought to play a key role in the regulation of hepatic fat metabolism and the secretion of triglyceride-rich lipoproteins. As with PNPLA3, it is thought to be located in lipid droplets and siRNA inhibition is associated with increased hepatocellular triglyceride concentration and lipid droplet lipid content

[84]. Variation in this gene has been shown to be associated with susceptibility to NAFLD independently of variation in PNPLA3, with the variant being identified as E167K or rs58542926 [85]. The allele frequency of this variant was shown to be 7.2% in European populations. A subsequent study of 361 individuals, including 226 patients with biopsy-proven NAFLD, has shown that this variant has a modest effect on NAFLD susceptibility and is associated with a slightly higher risk of developing NASH [86]. A further study of 1074 individuals demonstrated an association between this variant and advanced fibrosis and cirrhosis that occurred independently of potential confounding factors such as age, BMI, presence of type 2 diabetes and PNPLA3 genotype status [87]. However, it should be noted that 2 studies looking at this variant in Japanese [88] and Chinese [89] populations of individuals with biopsy-proven NAFLD failed to show an association between it and fibrosis stage or general histological severity. The Japanese study had relatively small numbers with 211 individuals and just 2 who were homozygous for E167K, and it should be noted that both of these studies focused on a single ethnic group that may not be directly applicable to other populations. A meta-analysis of 10 published studies looked at the relationship between the E167K variant and the presence of NAFLD in a total of 5537 study participants [90]. This revealed a carrier frequency of up to 7%, and demonstrated a moderate effect on the risk of developing NAFLD with an odds ratio of 2.13 (95% confidence interval 1.36-3.30). Crucially, it has been shown in a recent Finnish study that this variant is associated with preserved insulin sensitivity and a lack of hypertriglyceridaemia suggesting that this represents a distinct subtype of NAFLD similar to that associated with the PNPLA3 I148M variant [91]. **Figure 2** demonstrates the relationship between the 2 described genetic variants and the lipid droplet within the hepatocyte.

Figure 2 – Interaction between PNPLA3 and TM6SF2 variants and lipid metabolism in the hepatic lipid droplet. TAG; triacylglycerol, DAG; diacylglycerol, MAG; monoacylglycerol, VLDL; very low density lipoprotein, DGAT; diglyceride acyltransferase, ATGL; adipose triglyceride lipase, HSL; hormone sensitive lipase.



Further evidence for a dissociation between hepatic steatosis and insulin resistance may be found in the case of familial hypobetalipoproteinaemia (FHBL). Patients with FHBL have very low or absent levels of apolipoprotein B and this leads to an impairment of very low density lipoprotein export from the liver and consequently intra-hepatic accumulation of triglyceride. Amaro et al investigated a small number of overweight or obese patients with FHBL and observed that these individuals had greater insulin sensitivity than BMI- and hepatic triglyceride content-matched subjects with NAFLD alone [92]. The authors speculate that this would support the assertion that hepatic steatosis is a marker rather than a cause of the metabolic syndrome, however this was a very small study and it is not clear how applicable these findings are to the wider population of people with NAFLD. It has also been observed that lysosomal acid lipase deficiency (LAL-D), a rare autosomal recessive inherited condition, can lead to hepatic steatosis in the absence of metabolic syndrome [93].

There is also evidence that adipose triacylglycerol lipase (ATGL) may play a role in a potential dissociation between insulin resistance and hepatic steatosis [94]. ATGL acts to initiate hydrolysis of stored lipid by selectively cleaving triacylglycerols and not diacylglycerols or monoacylglycerols. Knock-out studies have demonstrated that ATGL-deficient mice experience a marked hepatic steatosis [95] and similarly overexpression of the ATGL gene leads to a reduction in liver fat in mice [96]. One study investigated the effects of ATGL gene manipulation on insulin sensitivity in mice, and here the authors observed that while ATGL knock-out mice do develop marked hepatic steatosis this does not result in any changes to their hepatocyte insulin sensitivity [97]. Hepatic ATGL overproduction in the same mice resulted in reduced hepatic steatosis, and interestingly the authors did observe a mild increase in insulin sensitivity although this was not sufficiently large to result in improvements in fasting glucose concentrations or insulinaemia.

Further insights into a possible disconnection between hepatic steatosis and insulin resistance can be gained by looking at disorders of fatty acid oxidation. In health, fasting stimulates gluconeogenesis in the liver fuelled by oxidation of fatty acids. If fatty acid oxidation is impaired this can lead to fasting hypoglycaemia and accumulation of lipids resulting in hepatic steatosis [98]. In such situations individuals will exhibit enhanced glucose tolerance, therefore exhibiting the disconnection. This occurs in numerous inborn errors of fatty acid oxidation such as medium chain acyl-CoA dehydrogenase deficiency (MCADD) and carnitine palmitoyl transferase II (CPT-2) deficiency [99]. Additionally, peroxisome proliferator-activated receptor alpha (PPARα) stimulates the expression of many genes involved in fatty acid oxidation. Experimental mice who have undergone PPARα knock-out develop marked hepatic steatosis after being exposed to a high fat diet, and after fasting demonstrate hypoglycaemia and increased insulin sensitivity [100].

Conclusion

It is clear from the literature that there is a complicated causal relationship between NAFLD and the metabolic syndrome. NAFLD is considered by many to represent the hepatic manifestation of the metabolic syndrome however rigidly sticking to this dogma does not appreciate the complexity of the relationship. Clearly the two clinical entities share many aspects of their pathophysiology, and insulin resistance is at the centre of both. There is sufficient evidence now for not only reciprocal causality between these disease states, but also each acting as a perpetuating or exacerbating factor for the other.

There are, however, many aspects of the interactions between NAFLD and the metabolic syndrome that are yet to be fully elucidated, and this is clearly demonstrated by the situations where there is an apparent disconnect or dissociation between them. Arguably, the hepatic steatosis that occurs in these situations due to genetic variation and inborn errors of metabolic can be considered a separate clinical entity to that which is associated with insulin resistance and the metabolic syndrome. However, focusing on the mechanisms that underlie these observations of dissociation could prove valuable for identifying new therapeutic targets in metabolic disease.

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Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "X.X. and Y.Y. conceived and designed the experiments; X.X. performed the experiments; X.X. and Y.Y. analyzed the data; W.W. contributed reagents/materials/analysis tools; Y.Y. wrote the paper." Authorship must be limited to those who have contributed substantially to the work reported.

PW and CDB conceived and designed the review. PW completed the first draft. CDB critically reviewed the draft and revised it with changes to the text and figures. PW and CDB completed the final draft and prepared it for submission.

Conflicts of Interest: All authors have no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:
NAFLD: Non-alcoholic fatty liver disease
PNPLA3: Patatin-like phospholipase domain-containing protein-3
TM6SF2: Transmembrane 6 superfamily member 2 protein
HDL-C: High density lipoprotein cholesterol
NASH: Non-alcoholic steatohepatitis
PKC-ε: Protein kinase C- ε
FLI: Fatty liver index
PAI-1: Plasminogen activator inhibitor-1
TNF-α: Tumour necrosis factor- α
IL: Interleukin
DAG: Diacylglycerols
DGAT: Diacylglycerol acyltransferase
SNP: Single nucleotide polymorphism
VLDL: Very low density lipoprotein
TAG: Triacylglycerol
MAG: Monoacylglycerol
ATGL: Adipose triglyceride lipase
HSL: Hormone sensitive lipase
FHBL: Familial hypobetalipoproteinaemia
BMI: Body mass index

References

1. Anstee QM, McPherson S, Day CP. How big a problem is nonalcoholic fatty liver disease? *BMJ* **2011**, 343: d3897.
2. Targher G, Byrne CD. Clinical review. Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *J Clin Endocrinol Metab* **2013,** 98: 483–95.
3. Chalasani N, Younossi Z, Lavine JE et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* **2012**, 55: 2005–23.
4. Browning JD, Szczepaniak LS, Dobbins R et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* **2004**, 40:1387-1395.
5. Aguilar M, Bhuket T, Torres S et al. Prevalence of the metabolic syndrome in the United States 2003-2012. *JAMA* **2015**, 313(19):1973-1974.
6. Cortez-Pinto H, Camilo ME, Baptista A, et al. Non-alcoholic fatty liver: another feature of the metabolic syndrome? *Clin Nutr* **1999**, 18:353–8.
7. Bedogni G, Miglioli L, Masutti F, et al. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* **2005**, 42:44–52.
8. Souza MR, Diniz Mde F, Medeiros-Filho JE, et al. Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. *Arq Gastroenterol* **2012**, 49:89–96.

9. Zhang Y, Zhang T, Zhang C et al. Identification of reciprocal causality between non-alcoholic fatty liver disease and metabolic syndrome by a simplified Bayesian network in a Chinese population. *BMJ Open* **2015**, 5:e008204.
10. Alberti KG, Eckel RH, Grundy SM et al. Harmonizing the metabolic syndrome. *Circulation* **2009**, 120:1640-1645.
11. Kim, HJ, Kim, HJ, Lee, K.E et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Arch. Intern. Med* **2004**, 164, 2169-2175.
12. Sinn, DH, Gwak, GY, Park, HN et al. Ultrasonographically detected non-alcoholic fatty liver disease is an independent predictor for identifying patients with insulin resistance in non-obese, non-diabetic middle-aged Asian adults. *Am J Gastroenterol* **2012**, 107, 561-567.
13. Targher G, Bertolini L, Padovani R et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* **2007**, 30:1212-8.
14. Leite NC, Salles GF, Araujo Antonio LE et al. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* **2009**, 29:113e9.
15. Assy N, Kaita K, Mymin D et al. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci* **2000**, 45:1929-34.
16. Lopez-Suarez A, Guerrero JM, Elvira-Gonzalez J et al. Nonalcoholic fatty liver disease is associated with blood pressure in hypertensive and nonhypertensive individuals from the general population with normal levels of alanine aminotransferase. *Eur J Gastroenterol Hepatol* **2011**, 23(11):1011–7.
17. Lau K, Lorbeer R, Haring R et al. The association between fatty liver disease and blood pressure in a population-based prospective longitudinal study. *J Hypertens* **2010**, 28(9):1829–35.
18. Marchesini G, Bugianesi E, Forlani G et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* **2003**, 37:917e23
19. Ryan MC, Wilson AM, Slavin J et al. Associations between liver histology and severity of the metabolic syndrome in subjects with nonalcoholic fatty liver disease. *Diabetes Care* **2005**, 28:1222e4
20. Day CP and James OF. Steatohepatitis: a tale of two ‘‘hits’’? *Gastroenterology* **1998**, 114:842-845
21. Tilg H and Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* **2010**, 52:1836-1846.
22. Samuel VT, Liu Z-X, Wang A et al. Inhibition of protein kinase Cε prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *J Clin Invest* **2007**, 117:739–745.
23. Kumashiro N, Erion DM, Zhang D et al. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *PNAS* **2011**, 108(39):16381-16385.
24. Choi JW, Wang, X, Joo, JI et al. Plasma proteome analysis in diet-induced obesity-prone and obesity resistant rats. *Proteomics* **2010**, 10, 4386–4400.
25. Meex RC, Hoy AJ, Morris A et al. Fetuin B Is a Secreted Hepatocyte Factor Linking Steatosis to Impaired Glucose Metabolism. *Cell Metabolism* **2015**, 22(6):1078-1089.
26. Hamaguchi M, Takeda N, Kojima T, et al. Identification of individuals with non-alcoholic fatty liver disease by the diagnostic criteria for the metabolic syndrome. *World Journal of Gastroenterology* **2012**, 18:1508–16.
27. Zhang T, Zhang Y, Zhang C et al. Prediction of metabolic syndrome by non-alcoholic fatty liver disease in northern urban Han Chinese population: a prospective cohort study. *PLos One* **2014**, 9(5): e96651.
28. Smits MM, Ioannou GN, Boyko EJ et al. Non-alcoholic fatty liver disease as an independent manifestation of the metabolic syndrome: results of a US national survey in three ethnic groups. *J Gastroenterol Hepatol* **2013**, 28: 664–670.
29. Adams LA, Waters OR, Knuiman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. *Am J Gastroenterol* **2009**, 104: 861–867.
30. Ryoo JH, Choi JM, Moon SY, Suh YJ, Shin JY, et al. The clinical availability of non alcoholic fatty liver disease as an early predictor of the metabolic syndrome in Korean men: 5-year’s prospective cohort study. *Atherosclerosis* **2013**, 227: 398–403.
31. Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol* **2007**, 102:2708–2715.
32. Mohan V, Farooq S, Deepa M, Ravikumar R and Pitchumoni CS. Prevalence of non-alcoholic fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and metabolic syndrome. *Diabetes Res Clin Pract* **2009**, 84: 84–91.

33. Rhoo JH, Suh YJ, Shin HC, Cho YK et al. Clinical association between non-alcoholic fatty liver disease and the development of hypertension. *J Gastroent and Hepatol* **2014**, 29:1926-31.
34. Rhoo JH, Ham WT, Choi JM, Kang MA et al. Clinical significance of non-alcoholic fatty liver disease as a risk factor for prehypertension. *J Korean Med Sci* **2014**, 29:973-979.
35. Huh JH, Ahn SV, Koh SB, Choi E et al. A prospective study of fatty liver index and incident hypertension: the KoGES-ARIRANG study. *PLoS ONE* **2015**, 10(11): e0143560.
36. Sung KC, Wild SH and Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. *J Hepatol* **2014**, 60:1040-1045.
37. Sung KC, Wild SH, Byrne CD. Resolution of fatty liver and risk of incident diabetes. *J Clin Endocrinol Metab* **2013**, 93(9):3637-43.
38. Vozarova B, Stefan N, Lindsay RS et al. High alanine Aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* **2002**, 51**:** 1889–95.
39. Lee DH, Ha MH, Kim JH et al. Gamma glutamyltransferase and diabetes – a 4 year follow-up study. *Diabetologia* **2003**, 46**:** 359–64.
40. Hanley AJ, Williams K, Festa A et al. Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. *Diabetes* **2005**, 54**:** 3140–7.
41. Ekstedt M, Franzén LE, Mathiesen UL et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* **2006**, 44**:** 865–73.
42. Monami M, Bardini G, Lamanna C et al. Liver enzymes and risk of diabetes and cardiovascular disease: results of the Firenze Bagno a Ripoli (FIBAR) study. *Metabolism* **2008**, 57**:** 387–92.
43. Goessling W, Massaro JM, Vasan RS et al. Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. *Gastroenterology* **2008**, 135**:** 1935–44.
44. Ryu S, Chang Y, Woo HY et al. Longitudinal increase in gamma-glutamyltransferase within the reference interval predicts metabolic syndrome in middle-aged Korean men. *Metabolism* **2010**, 59**:** 683–9.
45. Balkau B, Lange C, Vol S, Fumeron F, Bonnet F, Group Study D.E.S.I.R. Nine-year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study. *BMC Gastroenterol* **2010**, 10**:** 56–66.
46. Sung KC and Kim SH. Interrelationship between fatty liver and insulin resistance in the development of type 2 diabetes. *J Clin Endocrinol Metab* **2011**, 96**:** 1093–7.
47. Sung KC, Jeong WS, Wild SH and Byrne CD. Combined influence of insulin resistance, overweight / obesity, and fatty liver as risk factors for type 2 diabetes. *Diabetes Care* **2012**, 35(4):717-722.
48. Musso G, Gambino R, Cassader M, Pagano G. Meta analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* **2011**, 43**:** 617–49.
49. Ghosh AK and Vaughan DE. PAI-1 in tissue fibrosis. *J Cell Physiol* **2012**, 227(2):493-507.
50. Cesari M, Pahor M, Incalzi RA. Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions. *Cardiovasc Ther* **2010**, 28:e72–e91.
51. Oishi K. Plasminogen activator inhibitor-1 and the circadian clock in metabolic disorders. *Clin Expr Hypertension* **2009**, 31:208–219.
52. Bremer AA and Jialal I. Adipose tissue dysfunction in nascent metabolic syndrome. *J Obes* **2013**, 2013:393192.
53. Takeshita Y, TakamuraT, Hamaguchi E et al. Tumor necrosis factor-alpha-induced production of plasminogen activator inhibitor 1 and its regulation by pioglitazone and cerivastatin in a nonmalignant human hepatocyte cell line. *Metabolism* **2006**, 55(11):1464-72.
54. Baranova A, Gowder SJ, Schlauch K et al. Gene expression of leptin, resistin, and adiponectin in the white adipose tissue of obese patients with non-alcoholic fatty liver disease and insulin resistance. *Obes Surg* **2006**, 16:1118–25.
55. Baranova A, Schlauch K, Elariny H et al. Gene expression patterns in the hepatic tissue and in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. *Obes Surg* **2007**, 17(8):1111–8.
56. Hubscher SG. Histological assessment of non-alcoholic fatty liver disease. *J Clin Gastroenterol* **2006**, 40:S5–10.
57. Weltman MD, Farrell GC and Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. *Gastroenterology* **1996**, 111:1645–53.
58. Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. *Semin Liver Dis* **2001**, 21:3113–35.
59. Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam Horm* **2006**, 74:443–77.

60. You T, Nicklas BJ. Chronic inflammation: role of adipose tissue and modulation by weight loss. *Curr Diabetes Rev* **2006**, 2:29–37.
61. Henao-Mejia J, Elinav E, Cheng-Cheng J et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* **2012**, 482(7384):179-185.
62. Feldstein AE, Canbay A, Angulo P et al. Hepatocyte apoptosis and fas expression are prominent features of human non alcoholic steatohepatitis. *Gastroenterology* **2003**, 125: 437–443.
63. Bantel H, Ruck P, Gregor M and Shulze-Oshtoff K. Detection of elevated caspase activation and early apoptosis in lever disease. *Eur J Cell Biol* **2001**, 80: 230–239.
64. Civera M, Urios A, Garcia-Torres ML et al. Relationship between insulin resistance, inflammation and liver cell apoptosis in patients with severe obesity. *Diabetes Metab Res Rev* **2010**, 26:187-192.
65. Adams LA, Sanderson S, Lindor KD and Angulo P. The histological course of non-alcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* **2005**, 42:132-138.
66. El-Serag HB, Tran T and Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* **2004**, 126**:** 460–8.
67. Hamaguchi M, Kojima T, Takeda N et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann Intern Med* **2005**, 143**:** 722–8.
68. Porepa L, Ray JG, Sanchez-Romeu P, Booth GL. Newly diagnosed diabetes mellitus as a risk factor for serious liver disease. *CMAJ* **2010**, 182**:** E526–31.
69. Emerging Risk Factors Collaboration, Seshasai SR, Kaptoge S, Thompson A et al. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med* **2011**, 364**:** 829–41.
70. Graham RC, Burke A and Stettler N. Ethnic and sex differences in the association between metabolic syndrome and suspected nonalcoholic fatty liver disease in a nationally representative sample of US adolescents, *J Pediatr Gastroenterol Nutr* **2009**, 49(4):442-449.
71. Castro-Martinez MG, Banderas-Lares DZ, Ramirez-Martinez JC and Escobedode IPJ. Prevalence of nonalcoholic fatty liver disease in subjects with metabolic syndrome, *Cir Cir* **2012**, 80(2) 128-133.
72. Tsai CH, Li TC and Lin CC. Metabolic syndrome as a risk factor for non-alcoholic fatty liver disease. *South Med J* **2008**, 101(9) 900-905.
73. Zhang T, Zhang C, Zhang Y et al. Metabolic syndrome and its components as predictors of non-alcoholic fatty liver disease in a northern urban Han Chinese population: A prospective cohort study. *Atherosclerosis* **2015**, 240:144-148.
74. Monetti M, Levin MC, Watt MJ et al. Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metabolism* **2007**, 6 69-78.
75. Kantartzis K, Machicao F, Machann J et al. The *DGAT2* gene is a candidate for the dissociation between fatty liver and insulin resistance in humans. *Clin Sci* **2009**, 116:531-537.
76. Niebergall LJ, Jacobs RL, Chaba T and Vance DE. Phosphatidylcholine protects against steatosis in mice but not non-alcoholic steatohepatitis. *Biochim Biophys Acta* **2011**, 1811**:**1177–1185.
77. Jacobs RL, Zhao Y, Koonen DP et al. Impaired *de novo* choline synthesis explains why phosphatidylethanolamine *N*-methyltransferase-deficient mice are protected from diet-induced obesity. *J Biol Chem* **2010**, 285**,** 22403–22413.
78. Chamoun Z, Vacca F, Parton RG and Gruenberg J. PNPLA3/adiponutrin functions in lipid droplet formation. *Biol Cell* **2013**, 105(5):219-33.
79. Romeo S, Kozlitina J, Xing C et al. Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* **2008**, 40(12):1461-1465.
80. Romeo S, Sentinelli F, Dash S et al. Morbid obesity exposes the association between PNPLA3 I148M (rs738409) and indices of hepatic injury in individuals of European descent. *Int J Obes* **2010**, 34:190-194.
81. Romeo S, Sentinelli F, Cambuli VM et al. The 148M allele of the PNPLA3 gene is associated with indices of liver damage early in life. *J Hepatol* **2010**, 53:335-338.
82. Shen J, Wong GL, Chan HL et al. PNPLA3 gene polymorphism accounts for fatty liver in community subjects without metabolic syndrome. *Aliment Pharmacol Ther* **2014**, 39:532-539.
83. Kantartzis K, Peter A, Machicao F et al. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes* **2009**, 58:2616-2623.
84. Mahdessian H, Taxiarchis A, Popov S et al. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *PNAS* **2014**, 111(24):8913-8918.

85. Kozlitina J, Smagris E, Stender S et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* **2014**, 46:352–356.
86. Sookoian S, Castano GO, Scian R et al. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. *Hepatology* **2015**, 61:515-525.
87. Liu YL, Reeves HL, Burt AD et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* **2014**, 5:4309.
88. Akuta N, Kawamura Y, Arase Y et al. Relationships between genetic variations of PNPLA3, TM6SF2 and histological features of nonalcoholic fatty liver disease in Japan. *Gut Liver* **2015**, doi: 10.5009/gnl15163. [Epub ahead of print].
89. Wong VW, Wong GL, Tse CH, Chan HL. Prevalence of the TM6SF2 variant and non-alcoholic fatty liver disease in Chinese. *J Hepatol* **2014**, 61:708-709.
90. Pirola CJ and Sookoian S. The dual and opposite role of the TM6SF2-rs58542926 variant in protecting against cardiovascular disease and conferring risk for nonalcoholic fatty liver: a meta-analysis. *Hepatology* **2015**, 62:1742-1756.
91. Zhou Y, Llaurado G, Oresic M et al. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. *J Hepatol* **2015**, 62:657-663.
92. Amaro A, Fabbrini E, Kars M et al. Dissociation between intrahepatic triglyceride content and insulin resistance in familial hypobetalipoproteinemia. *Gastroenterology* **2010**, 139(1):149-153.
93. Reiner Z, Guardamagna O, Nair D et al. Lysosomal acid lipase deficiency – an under-recognized cause of dyslipidaemias and liver dysfunction**.** ***Atherosclerosis*** **2014**, 235:21-30.
94. Stefan N, Staiger H and Haring HU. Dissociation between fatty liver and insulin resistance: the role of adipose triacylglycerol lipase. *Diabetologia* **2011**, 54:7-9.
95. Haemmerle G, Lass A, Zimmermann R et al. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science* **2006**, 312:734–737.
96. Reid BN, Ables GP, Otlivanchik OA et al. Hepatic overexpression of hormone-sensitive lipase and adipose triglyceride lipase promotes fatty acid oxidation, stimulates direct release of free fatty acids, and ameliorates steatosis. *J Biol Chem* **2008**, 283:13087–13099.
97. Turpin SN, Hoy AJ, Brown AD et al. Adipose triacylglycerol lipase is a major regulator of hepatic lipid metabolism but not insulin sensitivity in mice. *Diabetologia* **2011**, 54:146-156.
98. Bennett MJ. Pathophysiology of fatty acid oxidation disorders. *J Inherit Metab Dis* **2010**, 33:533-537.
99. Sun Z and Lazar MA. Dissociating fatty liver and diabetes. *Trends Endocrinol Metab* **2013**, 24(1):4-12.
100. Kersten S, Seydoux J, Peters JM et al. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest* **1999**, 103:1489–1498.