Symposium on ‘Over-nutrition: consequences and solutions’

Session 2: Non-alcoholic Fatty Liver Disease: The Hepatic Consequence of Obesity and the Metabolic Syndrome

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Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase, MRI, magnetic resonance imaging; $^1$H MRS, proton magnetic resonance spectroscopy; ROS, reactive oxygen species; SREBP1, sterol regulatory element-binding protein-1; ChREBP, carbohydrate responsive element-binding protein; NHANES III, Third National Health and Nutrition Examination Survey; SNP, single nucleotide polymorphism; PNPLA3, patatin-like phospholipase domain-containing protein 3; SIBO, Small intestinal bacterial overgrowth; ATP III, Adult Treatment Panel III;
Abstract

Non-alcoholic fatty liver disease (NAFLD) is now the most common liver disease in both adults and children worldwide. A disease spectrum, NAFLD progresses from simple steatosis to steatohepatitis, advanced fibrosis and cirrhosis. An estimated 20-35% of the general population has steatosis and 10% of these will develop the more progressive non-alcoholic steatohepatitis associated with significant increased risk of cardiovascular- and liver-related mortality. Development of NAFLD is strongly linked to components of the metabolic syndrome including obesity, insulin resistance, dyslipidaemia and type 2 diabetes. The recognition that NAFLD is an independent risk factor for cardiovascular disease is a major public health concern. There is tremendous need for a sensitive, non-invasive test for early detection and staging of NAFLD that could also be used to monitor response to treatment. The cellular and molecular aetiology of NAFLD is multi-factorial; genetic polymorphisms influencing NAFLD have been identified and nutrition is a modifiable environmental factor influencing NAFLD progression. Weight loss through diet and exercise is the primary recommendation in the clinical management of NAFLD. The application of systems biology to the identification of NAFLD biomarkers and factors involved in NAFLD progression is an area of promising research.
Introduction

Non-alcoholic fatty liver disease is the pathological accumulation of fat in the liver in the absence of alcohol intake. Described initially only 30 years ago (1, 2), it is now the leading cause of liver disease in developed countries with an estimated prevalence of 20-30% in the general population. The occurrence of non-alcoholic fatty liver disease (NAFLD) is strongly linked to obesity, insulin resistance and other components of the metabolic syndrome. From a nutrition and public health perspective, the two major concerns are the rising incidence of NAFLD in children, and the convincing evidence that NAFLD is an independent risk factor for cardiovascular disease.

The term NAFLD encompasses a spectrum of histologically defined liver disorders. Disease can progress from macrovesicular lipid accumulation in the hepatocyte known as steatosis, to non-alcoholic steatohepatitis (NASH), steatosis in the presence of inflammatory infiltrate possibly with some fibrosis, to outright fibrosis, cirrhosis and even hepatocellular carcinoma. A combination of environmental and genetic factors determines individual risk of NAFLD development and progression, with a clear role for nutrition as a modifiable environmental risk factor. Pathogenesis of NAFLD was initially envisaged as a “two-hit process” (3) with fat accumulation in hepatocyte viewed as the primary insult and increased oxidative stress leading to inflammation being the second “hit” in progression to NASH and fibrosis. At the cellular level however, mechanisms influencing disease progression are clearly multi-factorial and dependent on numerous genetic and environmental interactions. Future analysis and modeling of NAFLD progression must account for this complexity in a systems biology fashion.

Diagnosis

The majority of NAFLD cases are identified after an incidental finding of either elevated liver enzymes on routine blood tests or suspected fatty liver on abdominal imaging in patients consuming little or no alcohol. (4) After the exclusion of excessive alcohol consumption which is generally defined as more than 20 g/day in men and 10 g/day in women, alternate causes of fatty liver (nutritional, genetic, viral, metabolic, drug) must also be excluded prior to diagnosis of NAFLD. While liver enzymes such as alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT) levels may be found elevated, they are not specific to NAFLD and furthermore the full histological spectrum of NAFLD has been observed in patients with normal ALT levels. (5) Non-invasive imaging techniques used for diagnosis of NAFLD include ultrasound, computed tomography, magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (1H MRS), each with its own set of advantages and disadvantages. (6) While all these techniques yield information about fat distribution in the liver, MRI and 1H MRS can quantify the
total amount of fat in the liver. In a population study using $^1$H MRS to assess liver fat, almost a third of subjects had hepatic steatosis and 79% of those with steatosis had normal ALT levels.\(^{(7)}\) These data highlight the concern that NAFLD prevalence as assessed by liver enzyme alterations is grossly underestimated.

Ultrasound-based transient elastography and magnetic resonance elastography are currently being investigated for their ability to stage liver fibrosis.\(^{(8, 9)}\) However a BMI greater than 28 was shown to cause transient elastography to fail\(^{(10)}\), and magnetic resonance elastography remains experimental.\(^{(11)}\) Although algorithms combining biochemical markers and patient parameters have been developed for predicting steatosis\(^{(12)}\), steatohepatitis\(^{(13)}\) and fibrosis\(^{(14)}\) in NAFLD, these require independent population validation and still lack sensitivity and specificity for widespread use. Histological assessment of liver biopsies remains the gold standard for diagnosing and staging NASH. However, liver biopsy is invasive and associated with significant morbidities\(^{(15)}\) and rare cases of mortality\(^{(16)}\) precluding its routine use in screening for NAFLD or for repeated assessment of either disease progression or response to therapy. Furthermore, as histological lesions are not evenly distributed in the liver, considerable sampling error and misdiagnosis exists.\(^{(17)}\) Consequently, the identification and validation of noninvasive biomarkers capable of diagnosing and staging NAFLD is a research priority.\(^{(18)}\)

**Epidemiology**

The lack of an inexpensive, noninvasive screening test for fatty liver disease means the true population prevalence for NAFLD remains unknown. Evidence from autopsy\(^{(19, 20)}\) and imaging\(^{(7, 21, 22)}\) studies demonstrates NAFLD prevalence in 20-35% of populations worldwide with 10% of these cases being NASH. Prevalence is much higher among the obese\(^{(20, 23-27)}\) and type 2 diabetics\(^{(28-30)}\) where NAFLD is found in 70-80% of patients, and advanced disease, NASH and fibrosis, is found in 25-70% of these cases. Distinct ethnic differences in the prevalence of NAFLD exist\(^{(7, 31)}\). The largest multiethnic population study to date with 2,287 subjects\(^{(7)}\), found the highest frequency of NAFLD in Hispanics (45%) compared to whites (33%) and blacks (24%); a gender difference was only observed in Caucasians (42% men, 24% women).

Shockingly, NAFLD is now the most common cause of liver disease in children. Incidence of paediatric NAFLD has risen sharply in the last three decades corresponding with the worldwide increase in childhood obesity. A retrospective review of paediatric autopsy reports in the United States found fatty liver in 13% of children and 38% of obese children between 2 and 19 years old.\(^{(32)}\) Estimates of NAFLD prevalence in obese children using ultrasonography range from 45-60%\(^{(33-36)}\), though no survey has been carried out in a UK population. However, the International
Obesity Task Force has concluded the lowest estimated prevalence of hepatic steatosis to be 27.9% among obese children in the European Union. Of particular concern are very recent datasets showing that NAFLD in overweight and obese children is not only strongly associated with cardiovascular risk factors, but also atherosclerosis as measured by carotid intima-media thickness. Since 7% of boys and 10% of girls aged 5-18 in the United Kingdom were obese in 2006 (28% and 36% overweight respectively), paediatric NAFLD warrants significant clinical and research attention.

**NAFLD development**

The pathogenesis of NAFLD begins with accumulation of lipid in the liver. As only a minority of patients with hepatic steatosis progress to the necroinflammatory steatohepatitis and develop fibrosis, Day originally conceptualized that a second “hit” was required to induce cellular events (e.g. oxidative stress) leading to inflammation, cell death and fibrosis. As with all complex diseases, it is now recognized that the phenotypic expression of NAFLD depends on a myriad of genetic, behavioral and environmental interactions.

While hepatic steatosis can be simplistically viewed as an imbalance between the processes of lipid accumulation and lipid disposal, the cellular and molecular regulation of hepatic metabolism is intricate and has been the subject of a recent extensive review. Fat in the liver is acquired from the diet, de novo lipogenesis or circulating NEFA and removed through VLDL secretion or by β-oxidation. Derangements in each of these pathways have been associated with NAFLD. Traditionally it appeared that lipid accumulation in the liver occurred in the context of obesity and peripheral insulin resistance, with lipid coming from an elevated plasma NEFA pool caused by increased activity of hormone-sensitive lipase and increased lipolysis from engorged adipocytes. Multiple lines of evidence suggest this explanation is likely to be too naïve; indeed it has been postulated that systemic insulin resistance might be secondary to NAFLD.

Stable isotope research in NAFLD patients has shown that, although 60% of the TAG in the liver comes from the peripheral NEFA pool, elevations in fasting de novo lipogenesis also contribute significantly. Additional work has observed both an increase in adipose lipolysis, and increased VLDL-TAG secretion from the liver in NAFLD patients. However, not only was the increase in VLDL-TAG secretion not sufficient enough to deal with the increase in hepatic TAG content in these patients, the VLDL-TAG was derived predominantly from “nonsystemic” NEFA, either from de novo lipogenesis or lipolysis of visceral fat. This is interesting in the light of very
recent ¹H-MRS and MRI data showing that hepatic lipid content directly correlates to visceral fat levels.⁴⁶ This study highlights ethnic differences in the manifestations of insulin resistance; although Hispanics and African Americans have similar frequencies of insulin resistance, African Americans are less likely to have NAFLD, hypertriglyceridaemia and elevated visceral fat depots. These data, as well as data showing NAFLD in nonobese individuals with normal glucose and lipid levels⁴⁷, mean NAFLD can and does occur without the backdrop of insulin resistance.

Once steatosis is established, multiple mechanisms contribute to lipid-induced cellular injury.⁴⁸ Increased mitochondrial β-oxidation of NEFA leads to an increase in reactive oxygen species (ROS). Mitochondrial defects have been observed in NAFLD patients and impairment of the respiratory chain also produces ROS and leads to extra-mitochondrial NEFA oxidation in the peroxisomes and microsomes producing further ROS. The overall increase in oxidative stress leads to lipid peroxidation, DNA and protein damage and ultimately cell death. In addition the aldehyde byproducts of lipid peroxidation increase production of proinflammatory cytokines and recruit inflammatory cells into the liver. Inflammation and the activation of hepatic stellate cells leads to collagen production and the initiation of fibrosis.

Lastly, the pathogenesis of NAFLD is also mediated by transcription factors such as the sterol regulatory element-binding protein-1 (SREBP1), the carbohydrate responsive element-binding protein (ChREBP) and the PPARs which are reviewed elsewhere⁴⁹, and influenced by circulating cytokines and adipokines. Adding additional complexity, the levels of all these molecules will depend on both genetic polymorphisms and the presence or absence of disease (e.g. obesity or immune). Clinical studies reviewed by Tsochatzis and colleagues⁵⁰ have shown low levels of the anti-inflammatory adiponectin and high levels of the pro-inflammatory resistin, TNF-α and IL-6 cytokines in NAFLD patients. Genetic polymorphisms in these cytokines have also been investigated in NAFLD patients as described later.

**NAFLD progression and associated mortality**

The prognosis of patients with NAFLD depends on the histological stage of the disease. Simple steatosis may have a relatively benign course with no increased risk of mortality, whereas NASH may progress quite rapidly and is associated with significant increased risk of mortality.⁵¹-⁵⁴ Progression in NAFLD is difficult to assess, requiring years of follow up and repeat biopsies which are prone to sampling errors.¹⁷,⁵⁵ The longest prospective study of the natural history of NAFLD using repeat biopsies had a mean follow-up of 13.7 years and 129 patients⁵⁴. This study showed
that 47% of patients presenting with steatosis progressed to NASH and 25-50% of patients presenting with NASH developed advanced fibrosis or cirrhosis in 8-13 years (Fig. 1). Although it underscores the fact that patients with steatosis can and do develop steatohepatitis, mortality was not changed in the group presenting with steatosis, whereas survival was significantly lower in patients that had presented with NASH (70% versus 80% in reference population, P = 0.01). Patients with NAFLD associated cirrhosis have been shown to have a very poor 10 year prognosis, with 50% needing a liver transplant, 20% dying from a liver-related cause and 7% developing hepatocellular carcinoma. Bleakly, a very recent study on the natural history of NAFLD in children demonstrates that paediatric NAFLD is also progressive and can result in end stage liver disease and death. In this cohort of 66 children followed over a mean of 6.4 years, 2 children underwent liver transplantation and 2 children died, while observed serial biopsies showed progression in 80% of patients.

Multiple risk factors have been associated with NAFLD progression including older age, BMI, insulin resistance, diabetes, metabolic syndrome, and histological NASH upon diagnosis. A current systematic review of ten studies examined risk factors for progression from NASH to advanced fibrosis. Using multivariate analysis this study found only age and presence of inflammation on initial biopsy to independently predict progression in 221 patients over a 5.3 year follow-up. The well documented limitations associated with systematic reviews, particularly the choice and heterogeneity of included studies in this case, likely explain why obesity, BMI and diabetes were not statistically significant predictors in this analysis.

Data regarding the mortality risks associated with NAFLD are somewhat conflicting. The first population-based cohort study examining NAFLD and mortality determined survival among 420 NAFLD patients, identified through imaging or biopsy, after a mean follow-up of 7.6 years. In agreement with case series from specialist clinics, NAFLD was found associated with significantly lower survival and increased risk of mortality from liver disease. After 10 years follow-up (n=161), survival was 77% versus 87% in the general population. Subsequently 3 independent groups have analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III) of the United States for mortality risk among suspected NAFLD cases based on elevated ALT levels. While both Ong and Dunn found NAFLD associated with an increase in overall mortality, Ong found NAFLD associated with a huge increase in liver associated mortality, whereas Dunn found NAFLD among 45-54 year olds dramatically linked to cardiovascular disease (CVD) mortality. Conversely, Ruhl did not find elevated ALT levels associated with all-cause or CVD mortality but did find a significant association with liver related mortality. Differences are likely explained by variations in study design, patient selection strategy.
and statistical analysis. While these studies have the advantage of very large samples sizes from a representative sample of the general population, diagnosis of NAFLD is based on elevated ALT levels which do not differentiate between steatosis and steatohepatitis and, as previously mentioned, are likely to severely under assess NAFLD incidence and therefore the magnitude of mortality risk. Regardless, the data in total show NAFLD is far from a benign diagnosis.

**Genetic and environmental factors associated with NAFLD development and progression**

The development of NAFLD is strongly linked to obesity and insulin resistance. However, as there are obese and diabetic individuals who do not have NAFLD, and since NAFLD can occur in normal weight individuals with normal glucose and lipid levels\(^{(47)}\), there are obviously multiple genetic and environmental factors determining NAFLD development and progression (Fig. 2).

Initial evidence for a genetic component to NAFLD comes from familial clustering studies\(^{(69, 70)}\) and the ethnic variation in NAFLD prevalence\(^{(7)}\). Various genetic single nucleotide polymorphisms (SNPs) have been investigated in NAFLD including SNPs in the adiponectin\(^{(71, 72)}\), IL-6\(^{(73)}\), TNF-\(\alpha\)\(^{(74)}\), and apolipoprotein E\(^{(75)}\) genes among others. Investigated in a “candidate gene” fashion in small cohorts, these studies have yielded somewhat conflicting results in different populations. In 2008 the first genome-wide association scan in a large multiethnic population targeting NAFLD, identified the PNPLA3 (Patatin-like phospholipase domain-containing protein 3, also known as adiponutrin) gene strongly associated with hepatic triglyceride content.\(^{(76)}\) Allele variants of the PNPLA3 gene associated with high and low amounts of hepatic fat were found enriched in Hispanics and African-Americans respectively, correlating with the high and low prevalence of NAFLD in these populations.\(^{(7)}\) Remarkably, PNPLA3 was also independently identified in a separate population-based genome-wide scan as one of two genetic loci influencing plasma levels of ALT.\(^{(77)}\) Undoubtedly as genome sequencing continues to get cheaper and faster, studies of this type will reveal more genes causal in NAFLD pathogenesis.

Nutrition and physical activity are important environmental factors determining risk of NAFLD. Excess food intake and lack of exercise contribute to weight gain which has been shown to contribute to the progression of liver fibrosis in NAFLD patients.\(^{(54)}\) Specific dietary factors may also play either protective or antagonistic roles in the development and progression of NAFLD. Assessment of nutrient intakes in NAFLD has been done in Italian\(^{(78)}\), Japanese\(^{(79)}\), Israeli\(^{(80)}\) and US\(^{(81)}\) populations; no UK cohort has been assessed to date. Increased consumption of meat and soft drinks and low consumption of fish were associated with NAFLD cases as opposed to control.
Unsurprisingly, low intakes of PUFA and high intakes of saturated fat and cholesterol were also associated with NAFLD. Data from two randomised controlled trial have shown beneficial effects from six months dietary supplementation with n-3 PUFA in NAFLD compared to dietary advice alone. (82, 83) Reductions in fatty liver observed by ultrasonography as well as improvements in serum ALT and triglyceride levels were significantly greater in those supplemented with n-3 PUFA. In both trials the patients receiving PUFA were more likely to have complete fatty liver regression suggesting a beneficial therapeutic effect for n-3 PUFA supplementation in NAFLD. Other studies have found higher carbohydrate and lower fat diets associated with more progressive disease. (84, 85) Conversely, very recent animal data alarmingly shows that, in both mice (86) and nonhuman primates (87), exposure to a maternal high fat diet leads to development and progression of NAFLD in the offspring.

Given the role of oxidative stress in NAFLD pathogenesis several studies have examined antioxidant supplementation as an intervention in NAFLD. A recent Cochrane review of six very small and extremely diverse clinical trials did not find enough data to recommend either for or against the use of antioxidant supplements in NAFLD patients. (88) However, data from pilot studies (89, 90) and one double-blind, randomized, placebo-controlled trial (91) have shown sufficient positive effects with Vitamin E supplementation in NAFLD patients that this supplement is now the subject of two ongoing multicenter trials. The “Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Non-alcoholic Steatohepatitis” (PIVENS) trial (92) in adults and the “Treatment of Non-alcoholic Fatty Liver Disease in Children” (TONIC) trial (93) both involve 96 weeks of treatment and will measure improvements in histology as well as ALT levels. While the PIVENS trial has 3 arms and includes a placebo group, the TONIC trial in children will compare metformin treatment to Vitamin E supplementation. Data from these trials, whether negative or positive, should end the debate on efficacy of Vitamin E supplementation in NAFLD.

Small intestinal bacterial overgrowth (SIBO) may be an additional environmental factor involved in NAFLD pathogenesis where dietary supplements such as probiotics could have a beneficial effect. Evidence from animal studies shows that SIBO increases gut permeability leading to portal endotoxaemia and increased circulating inflammatory cytokines both of which have been implicated in the progression of NAFLD. (94) Several reports have found SIBO associated with progressive NAFLD (95-97) and in one small human study, treatment with antibiotics resulted in an increase in fasting insulin levels. (97) Limited evidence from animal models and one preliminary human study suggest supplementation with the probiotic VSL#3 may possibly be beneficial in NAFLD, but data is mixed. (98-100) More research in this area is warranted.
NAFLD, the metabolic syndrome and CVD risk

The metabolic syndrome was originally defined as a cluster of CVD risk factors with insulin resistance considered the underlying pathogenic factor.\textsuperscript{(101)} The third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATP III]) defines metabolic syndrome as being any three or more of five variables: increased waist circumference, hypertriglyceridaemia, hypertension, low HDL cholesterol, and hyperglycaemia.\textsuperscript{(102)} Metabolic syndrome is highly prevalent among NAFLD patients in multiple populations and is strongly associated with progressive disease.\textsuperscript{(61, 103-105)} Equally, a Japanese prospective study (N=3147) demonstrated that patients with metabolic syndrome, as defined by ATP III, were 4 to 11 times (men and women respectively) more likely to have developed NAFLD upon follow-up.\textsuperscript{(106)} Likewise, children with metabolic syndrome as defined by ATP III have been shown to be 5 times more likely to have NAFLD.\textsuperscript{(38)} This strong relationship between NAFLD and the metabolic syndrome has led to the description of NAFLD as the hepatic component of the metabolic syndrome.

Indeed, in the last five years NAFLD has emerged as an independent risk factor for CVD. Several studies have observed increased carotid intima-media thickness (IMT) and carotid plaques in NAFLD patients, including children.\textsuperscript{(39, 107-111)} Although there has been some conflicting work\textsuperscript{(112)}, a systematic review found that carotid plaques were 2 to 3 times more likely to be observed in NAFLD patients and IMT was strongly associated with NAFLD.\textsuperscript{(113)} Furthermore, multiple population studies have observed increased CVD events in NAFLD patients diagnosed by ultrasonography and demonstrated that the increased risk for CVD in NAFLD is independent of conventional risk factors and components of the metabolic syndrome.\textsuperscript{(114-116)} These data add to population data where elevated liver enzymes have also been associated with increased risk for CVD.\textsuperscript{(67, 117)}

Essentially as the treatment of NAFLD involves correcting the same metabolic factors involved in CVD, it is prudent that all patients with NAFLD be evaluated for early atherosclerosis. Likewise, patients presenting with the metabolic syndrome or a high Framingham risk score\textsuperscript{(118)} should be evaluated for the presence of NAFLD. Children identified with NAFLD or the metabolic syndrome should be strongly counseled to avoid smoking, increase their physical activity and improve their nutrition in order to prevent the development of cardiovascular disease.

The potential of systems biology
Systems biology is the study of a biological system (cell, tissue, organism) viewed as an integrated and interacting network of genes, proteins and biochemical reactions. This methodology combines computational modeling with experimental biology in order to discover emergent properties that arise from studying a system as a whole; it is these properties that ultimately determine how a system is controlled or regulated. In a systems approach one begins by constructing a predictive model from which a hypothesis is formed and then tested experimentally by perturbing the system. Experimental techniques are generally the global approaches of genomics, proteomics and metabolomics. Data obtained from these multiple approaches are integrated and compared to the original model, which is refined in an iterative process. In the context of human health it is believed that systems biology is essential to future drug discovery and indeed, to a future of personalized, predictive and preventative medicine. In this vision, drug targets would be rational and identified through the analysis of normal and disease perturbed networks, while an individual could have their genome sequenced and in combination with extensive blood analyses would be able to obtain a “probabilistic future health history”. Although systems biology is in its infancy and this vision still seems far off, global approaches have been applied to NAFLD and these datasets are relevant to the future creation of an integrated “systems” model of NAFLD.

Proteomic technologies are still evolving and analysis of a complete proteome, in the manner of DNA microarray analysis of complete genomes, has yet to be realized. However, as disease biomarkers and drug targets are most likely to be proteins, proteomics is viewed as central to systems biology and key to predictive medicine. In the context of NAFLD, proteomic techniques have been used to analyze adipose, liver and most recently serum samples in NAFLD patients. Ultimately it is most likely that a serum diagnostic or prognostic tool for NAFLD would be comprised of a panel of proteins, and this research highlights the power of systems methodologies for identifying candidate protein biomarkers. Significantly in the study by Bell and colleagues their “potential biomarker panel” comprising of 6 proteins demonstrated greater diagnostic utility than ALT to differentiate between their patient groups. It is also noteworthy that Charlton and Bell independently identified lumican, a protein that activates transforming growth factor-beta and smooth muscle actin, elevated in both liver and serum from NASH patients. These studies have given insight into NAFLD pathogenesis and have identified candidate disease biomarkers worthy of prospective validation.

Conclusion
Non-alcoholic fatty liver disease is highly prevalent with an estimated 20-30% of the UK population affected. Research on the natural history of NAFLD has demonstrated that even simple steatosis cannot be considered benign as 47% of these patients will progress to NASH in 8-13 years. An estimated 25-50% of patients with NASH will progress to advanced fibrosis and cirrhosis. In real numbers this is 300,000 to 1.8 million cases in the UK, 50% of whom will require a liver transplant to avoid death. It is clear that a diagnosis of NAFLD is associated with increased risk of mortality from liver- and cardiovascular-related events and the British Liver Trust has demonstrated that liver disease is the only major cause of death still increasing yearly in the UK.\(^{(124)}\) An additional public health concern is the rising incidence of NAFLD in children who are not exempt from risk of cardiovascular disease, the need for liver transplants or early death. Part of the challenge in assessing the burden of NAFLD in the population is the lack of a non-invasive screening test for NAFLD.

While NAFLD incidence is strongly linked to insulin resistance and the metabolic syndrome, the development and progression of NAFLD involves a myriad of molecular and cellular events that are influenced by both genetic and environmental factors. Determination of the critical factors involved in disease progression will identify possible therapeutic targets for treatment of NAFLD and may form the basis of prognostic tests. Proteomic studies are beginning to identify serum and liver proteins found altered in NAFLD. The modeling of NAFLD protein and gene regulatory networks in a systems biology fashion is most likely to yield diagnostic, prognostic and therapeutic targets for NAFLD and more research of this type is urgently needed.

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111. Kim HC, Kim DJ & Huh KB (2009) Association between nonalcoholic fatty liver disease and carotid intima-media thickness according to the presence of metabolic syndrome. *Atherosclerosis* 204, 521-525.


Fig. 1. Progression of non-alcoholic fatty liver disease (NAFLD). 47% of patients presenting with steatosis progress to steatohepatitis and 25-50% of patients presenting with steatohepatitis develop fibrosis or cirrhosis within 8-13 years. The 10 year prognosis for patients with NAFLD-related cirrhosis is very poor with 50% requiring a liver transplant, 20% dying of a liver-related cause and 7% developing hepatocellular carcinoma. Dashed lines indicate the potential for reversal.
Fig 2. Genetic and environmental factors associated with non-alcoholic fatty liver disease (NAFLD) development and progression. Solid lines indicate that comorbidities (ovals) in addition to genetic and environmental factors influence the development and progression of NAFLD. Dashed lines show that genetic and environmental factors also influence the development of comorbid conditions.