Chapter 21

Non-alcoholic fatty liver disease (NAFLD) in diabetes: distraction or impending disaster?

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Introduction

Sedentary lifestyles coupled with excessive caloric intake have been major determinants to the explosive increase in the prevalence of obesity and type 2 diabetes mellitus (T2DM). Endocrinologists are encountering on a daily basis patients with obesity-related complications, such as metabolic syndrome (MetS) and T2DM. The epidemic of T2DM has driven health care providers to give increasing attention to the early diagnosis and treatment of diabetic complications. However, not until recently has non-alcoholic fatty liver disease (NAFLD) been recognized as another common complication of patients with T2DM that requires special attention. Reasons for this may be related to the lack of awareness about the increased susceptibility to fatty liver disease of patients with T2DM, inherent difficulties in making the diagnosis and incomplete understanding about the natural history of the disease. The management of patients with NAFLD has traditionally belonged to the realm of hepatologists, but realization that the majority of obese or T2DM patients may have a fatty liver has become a new and concerning medical dilemma for primary care physicians and endocrinologists. This chapter will briefly review our current knowledge about NAFLD trying to piece together our incomplete knowledge of the disease and weight into the controversy on whether this condition carries an independent and inherent health risk or it is simply an epiphenomenon of a state of insulin resistance and abnormal glucose metabolism.

Natural history

NAFLD and liver disease progression

NAFLD is a common cause of chronic liver disease, and its worldwide prevalence continues to increase with the obesity epidemic [1, 2]. Liver fat deposition may range from simple steatosis to severe steatohepatitis with hepatocyte necrosis, inflammation and variable degrees of fibrosis (non-alcoholic steatohepatitis or NASH). It is believed that in patients with NAFLD, ~40% or more may go on to develop NASH [3-5] **(III/B),** although the natural history of the disease is not well established.

Studies on the natural history of NAFLD have been challenging as they require paired liver biopsy samples, possibly the major determinant of the small nature of the available studies. Difficulties when interpreting the available studies in the NAFLD literature include:

- ◆ the inherent selection bias of work from tertiary health care facilities;
- ◆ variable length of follow-up across studies;
- ◆ histological interpretation, being inherently difficult and affected by liver biopsy sample variability as well as reading standardization among pathologists and between medical centers;
- ◆ incomplete information in published studies about the associated metabolic risk factors during follow-up that likely impact disease progression (i.e. weight gain/obesity, development of diabetes, diabetes control, other);
- ◆ inclusion of individuals with advanced disease (cirrhosis) at baseline; and
- ◆ the retrospective nature of most reports.

Despite these limitations, the available studies have provided useful insights regarding the progression and prognosis of the disease.

Table 1 summarizes the most relevant of these studies and suggests that NASH may progress to advanced fibrosis in a significant number of patients. Indeed, NASH is now recognized as a major cause of cryptogenic cirrhosis [6-9] **(IIb/B)**. In studies with paired liver biopsies with 3 years or more of follow-up [7, 10-13], the overall rate of progression of fibrosis was between 28% to 41%. A number of factors seem important on determining disease progression. Among them, disease duration is typically the most relevant factor, with more severe steatohepatitis and fibrosis reported in studies with longer follow-up [7]. Obesity (or weight gain over time), diabetes at baseline and advanced hepatic fibrosis on the initial liver biopsy [7, 11-13] were all consistent factors associated with disease progression and poor prognosis **(IIa/B)**. For instance, the role of disease duration is illustrated in a study by Adams *et al* [12] that reported that in patients with NASH followed with paired liver biopsies, disease progression was two-fold higher if follow-up was extended for up to 4 years compared to shorter periods of time. In the work by Ekstedt *et al* [7], 41% of patients had signs of progressive fibrosis after a mean follow-up of 13.8 years. Adding complexity to the individual risk assessment and management of NAFLD is the observation that a significant minority of patients – up to 18% in some series [10, 12] – may advance to cirrhosis within just 3 years.

Table 1. Natural history of NASH: disease progression in studies with paired liver biopsies.

Author (year)	n	Follow-up (mean years)	Disease progression*	Predictors
Harrison *et al* (2003)	22	5.7	32%	↑AST
Fassio *et al* (2004)	22	4.3[†]	32%	ALT not useful Obesity
Lindor *et al* (2004)	107	2.0	25%	AST and ALT not useful
Adams *et al* (2005)	103	3.2	37%	ALT not useful Obesity DM
Ekstedt *et al* (2006)	68[‡]	13.8	41%	↑AST, ALT DM, IR (by HOMA) LFAT
Wong *et al* (2010)	52[§]	3.0	28%	AST/ALT ratio not useful Obesity ↑LDL-C
Sanyal *et al* (2010)	247	2.0	19%	ALT not useful

AST = aspartate aminotransferase; ALT = alanine aminotransferase; DM = diabetes mellitus; IR = insulin resistance; HOMA = Homeostatic Model Assessment Index; LFAT = liver fat; LDL-C = low density lipoprotein cholesterol
* Progression to fibrosis
[†] Median
[‡] 88 out of 129 had follow-up data (but 68 had a second liver biopsy), at baseline only 55% NASH (4 of them had cirrhosis), 9% steatosis with unspecific inflammation and 36% simple steatosis
[§] 25% steatosis, 42% borderline NASH, 33% NASH

Dysfunctional adipose tissue, as observed in obesity, is perhaps the single most important risk factor for poor long-term outcomes. Most patients undergoing liver transplantation for cryptogenic cirrhosis are obese and the vast majority represent cases of undiagnosed NASH. In the study by Ekstedt *et al* [7], progression of liver fibrosis was most strongly associated with significant weight gain (>5kg) and insulin resistance. In our experience, the development of liver fibrosis is more dependent on the severity of adipose tissue dysfunction and plasma free fatty acid (FFA) concentration than the degree of obesity *per se*. Lomonaco *et al* [14] divided obese subjects into quartiles of adipose tissue insulin resistance (the product of fasting

plasma FFA x fasting plasma insulin concentration, a validated index of adipose tissue dysfunction in obese individuals) and reported that the severity of hepatic insulin resistance, atherogenic dyslipidemia and of liver fibrosis on histology was not directly related to BMI or visceral fat, but rather directly proportional to adipose tissue insulin resistance and hepatic lipotoxicity.

An aspect of these studies that has important clinical implications is the overall lack of correlation between plasma aminotransferases levels and changes in liver fibrosis over time. While some studies have found a weak correlation between liver disease and plasma aminotransferases [7, 10], most studies have not found them alone to be very useful in predicting disease severity or treatment response [11-13, 15]. However, in combination with common clinical risk factors such as obesity and diabetes, as well as liver imaging (i.e. steatosis on ultrasound), they may be helpful for assessing risk of NAFLD. For instance, elevated plasma aminotransferases in the presence of both high cholesterol and triglycerides (TG) was associated with NAFLD in 83% of patients in the Dallas Heart Study [16]. In some studies elevated liver aminotransferases were indicative of worse hepatic insulin resistance and overall prognosis in NAFLD [14, 17]. These abnormalities, combined with laboratory indicators of advanced liver disease (such as plasma low platelet count and elevated bilirubin), are clearly useful indicators of advanced liver disease but not very sensitive for an early diagnosis. The true dilemma is the diagnosis of NAFLD, and in particular of NASH, in the early stages of the disease when mild to moderate liver fibrosis is present. This is an important distinction because long-term prognosis of liver disease seems to go hand-in-hand with the development of steatohepatitis, and in particular, of fibrosis. In this setting, liver enzymes have been of disappointing diagnostic value [17-21], and when within the normal range, offer a false sense of security. Many recent studies report the presence of advanced NASH even with normal liver aminotransferases [8, 14, 22, 23], but may underestimate liver fat accumulation in patients with T2DM [24]. In summary, these findings have important implications in the care of patients with NAFLD. Although they could be indicative of severe metabolic disease and fatty liver infiltration, increased liver aminotransferases are poor guides for the overall management of patients with NAFLD and currently only a liver biopsy can accurately determine the severity of NAFLD and need for treatment.

NAFLD and long-term mortality

Whether NAFLD is associated with an increased risk of premature death is highly controversial, and at the present time, has no definitive answer. Again, studies have been plagued by shortcomings such as small sample size, patient selection bias, lack of standardized follow-up and the presence of overlapping cardiovascular risk factors typical of patients with NAFLD. In many studies, after the initial baseline diagnosis of NASH, no liver biopsy confirmed the severity of liver disease (or its resolution) at the end of the observation period. This is important because as many as ~18-25% of patients have improvement or resolution of NASH over time [7, 25-27]. Thus, it remains unclear whether the overall mortality reported in these studies was linked to liver disease or associated metabolic factors.

	Author	n	Follow-up (mean, yrs)	Liver-related mortality	Overall mortality	Increased Mortality? ‡
Table 2. Long-term liver-related and overall mortality in middle-aged patients with NAFLD.						
Simple steatosis	Matteoni et al (1999)	49	9	2.0%	33.0%	No
	Ekstedt et al (2006)	58	14	0.0%	12.1%	No
	Rafiq et al (2009)	74	19*	2.7%	56.8%	No
	Soderberg et al (2010)	67	21	3.0%	34.3%	No
	Dam-Larsen et al (2009)	170	21	0.6%	28.2%	No
	Total/mean	**418**	**17**	**1.7%**	**32.9%**	
NASH	Matteoni et al (1999)	29	8	10.0%	30.0%	Yes
	Ekstedt et al (2006)	71	14	2.8%	26.8%	Yes
	Rafiq et al (2009)	57	19*	17.5%	63.2%	Yes
	Soderberg et al (2010)	51	21	5.9%	47.1%	Yes
	Evans et al (2002)	26	9	NR	15.0%	Yes
	Adams et al (2005)	49	8	8.1%	35.0%	Yes
	Younossi et al (2011)	131	10*	15.7%	21.3%	NR
	Total/mean	**349**	**11**	**8.6%**	**34.1%**	
NAFLD-related cirrhosis	Hui et al (2003)	23	7	21.0%	26.0%	NR
	Sanyal et al (2006)	152	10	14.5%	19.1%	NR
	Yatsuji et al (2009)	68	5	7.3%	27.9%	NR
	Bhala et al (2011)	247	7	5.7%	13.4%	Yes
	Total/mean	**490**	**7**	**12.1%**	**24.3%**	

NR = not reported
‡ When compared to the general population
* Median

As summarized in Table 2, long-term liver-related prognosis is closely correlated with the severity of steatohepatitis, and in particular, of fibrosis **(IIa/B)**. Some studies have had follow-ups of ~20 years [25, 26, 28]. Patients with simple ('bland') steatosis appear to have a good long-term prognosis. They rarely die of liver-related complications and overall mortality does not appear to be increased, although only a handful of studies are available and they carry the limitations discussed above. In contrast, patients with steatohepatitis (NASH) have an approximately three-fold increase in liver-related mortality (3.6% vs. 8.6%; Table 2) [7, 12, 25, 26, 29-31]. Several studies [7, 25, 26], but not all [29], have suggested that patients with NASH have a higher overall mortality rate compared to patients with simple steatosis. In the study with the longest follow-up (mean duration of 21 years), Soderberg et al [26] reported a clear difference in the overall survival of subjects with NAFLD when divided among those with only fatty liver or subjects with fat and any type of inflammation, ballooning, or fibrosis (Figure 1). Mortality was also higher when patients with steatohepatitis (NASH) were compared to patients with
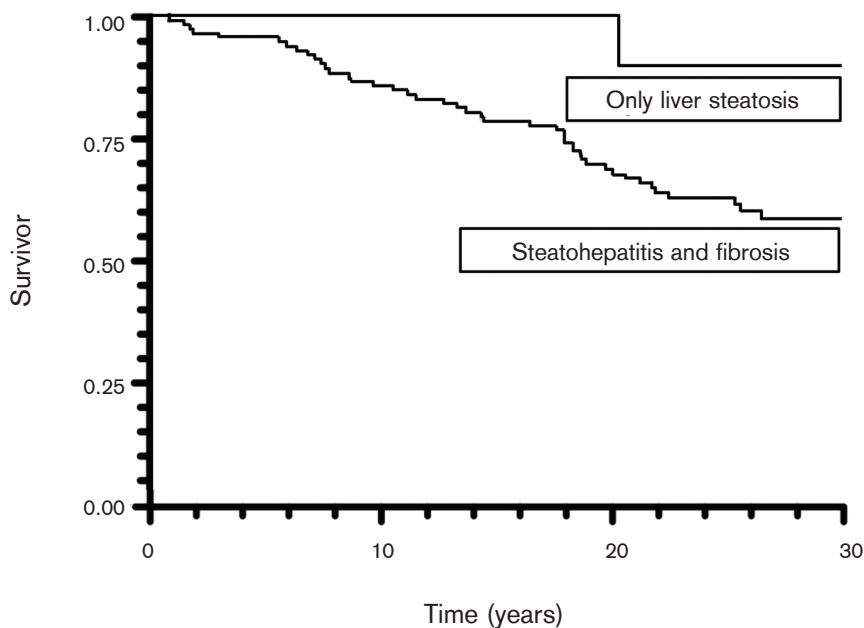

Figure 1. Overall survival of subjects with NAFLD, divided among those with only fatty liver or subjects with fat and any type of inflammation, ballooning, or fibrosis. *Adapted from Soderberg C, et al* [26]. *Reprinted with permission from John Wiley and Sons, © 2009.*

simple steatosis (47.1% vs. 34.3%). Ekstedt *et al* [7] found no liver-related mortality after 14 years of follow-up in patients with simple steatosis compared to 2.8% in patients with NASH and a 2.5-fold increase in overall mortality. If the data of all studies in Table 2 are taken together, it appears that after 11 years of follow-up patients with NASH even have a worse prognosis than patients with simple steatosis followed for 17 years, with a five-fold increase in liver-related mortality and higher overall mortality. What is unquestionable is that patients with NAFLD-related cirrhosis are at a much higher risk of liver-related and overall mortality, with 5-10 years liver-related mortality being as high as 12.1% and overall mortality 24.3% [32-35].

NAFLD in patients with type 2 diabetes mellitus

In the last decade, a number of epidemiological and clinical translational studies have led to an increased awareness about the close link between NAFLD and T2DM. Both conditions frequently coexist with severe hepatic insulin resistance playing a key role in the pathophysiology of the disease. Epidemiological studies reveal that elevated plasma aminotransferase levels are associated with a greater risk of having diabetes [36-41]. Moreover, even plasma alanine aminotransferase (ALT) within the 'normal' range is associated with higher diabetes incidence, independently of classical predictors (i.e. BMI, plasma glucose levels, presence of the MetS) [37, 42, 43]. The prevalence of abnormal glucose metabolism in patients with NAFLD based only on self-reported diagnosis or a fasting plasma glucose ranges between 9 to 31% [8, 44, 45]. However, when studies have systematically screened patients with NAFLD for impaired glucose tolerance (IGT) or T2DM with an oral glucose tolerance test (OGTT) the true rate of abnormal glucose metabolism is much higher. In our experience, when obese patients believed to have normal glucose metabolism (by both the patient and primary care provider) are systematically screened with an OGTT, the prevalence of prediabetes (impaired fasting glucose [IFG], IGT) and newly diagnosed T2DM is considerably higher in patients with NAFLD versus matched controls without a fatty liver (83% vs. 38%, p<0.001). Patients with NAFLD had an approximately three-fold higher rate of newly diagnosed T2DM [46]. Of note, the very high rate of abnormal glucose metabolism in this cohort was likely related to a high prevalence of known risk factors for the development of T2DM (such as high rates of sedentarism, obesity and Hispanic ancestry), but similar high prevalence rates of IGT and T2DM (ranging from 33% to 62%) have been recently reported in studies from China [47], Japan [48] and in a predominantly European population from Australia [49].

While T2DM is more common in NAFLD, the nature of this relationship is incompletely understood and it remains to be established if hepatic steatosis precedes the development of steatosis and directly contributes to the development of diabetes, or is an associated condition more closely mirroring the state of insulin resistance. Defects in adipose tissue are well established in obesity and T2DM [50, 51]. The pathogenesis of NAFLD in the setting of insulin resistance involves a cross-talk between adipose tissue and target tissues such as the liver and skeletal muscle [50, 52]. Oversupply of free fatty acids (FFA) to the liver from insulin resistant adipose tissue and a state of local (hepatic) and systemic chronic inflammation are at center stage in the development of steatosis and NASH [14, 18, 53, 54]. In the setting of excessive FFA supply, both chronic hyperglycemia and hyperinsulinemia promote hepatic triglyceride synthesis and increase the secretion of VLDL. However, it must be recognized

that although *de novo* lipogenesis (DNL) is increased in NAFLD, it contributes less to the total hepatic triglyceride pool (~20-25%) than fatty acids from adipose tissue (~60%) in NAFLD [55]. Oversecretion of VLDL may be an adaptation to prevent massive steatosis and may explain why so frequently high plasma triglycerides, low high-density lipoprotein (HDL-C), and increased formation of small, dense low-density lipoprotein (LDL-C) are so common in patients with MetS, T2DM and NAFLD [46, 56]. In patients with steatosis there is a failure of insulin to suppress VLDL secretion [57, 58] and secretion rates of the lipoprotein increase in proportion to the severity of intrahepatic triglyceride accumulation [59]. Resistance to insulin's action to suppress glucose and VLDL secretion in patients with T2DM may account for the observation that diabetes control may be more difficult to achieve and require higher insulin doses in patients with T2DM and NAFLD [60]. However, hepatic steatosis may be ameliorated and plasma triglycerides improved by good glycemic control in patients with T2DM and NAFLD (Cusi *et al*, unpublished).

T2DM is also associated with worse liver disease although the underlying mechanisms remain unclear [8, 54]. For instance, in epidemiological studies T2DM is associated with a two- to four-fold increase in advanced liver disease [3, 7], cirrhosis [4] and hepatocellular carcinoma [4, 9, 61, 62] **(IIb/B)**. The presence of T2DM in patients with NAFLD is associated with more severe hepatic and adipose tissue insulin resistance [14, 18, 46] and much worse liver histology, including frequently advanced liver fibrosis or cirrhosis [2, 6-8, 10, 11, 14, 18, 23, 41, 46, 63]. However, well-controlled long-term prospective studies on the natural history of NAFLD in T2DM are lacking.

In summary, the available evidence calls for endocrinologists to be aware about the possible role of NAFLD in patients with T2DM. While the evidence is still limited, the data reviewed suggests that the presence of NAFLD may play a pathogenic role, or at least be a hallmark of, future T2DM. It may also identify a subset of patients with more severe hepatic insulin resistance that are more prone to the development of dyslipidemia and at risk of earlier oral agent failure. Finally, the association of T2DM and NASH places these patients at an increased risk of severe liver disease, including hepatocellular carcinoma. Taken together, there is a compelling need to develop non-invasive ways to establish an early diagnosis of NASH in patients with T2DM, or in selected patients, perform a liver biopsy (i.e. those with more risk factors for NASH, liver aminotransferases >three-fold upper limit of normal, clinical or imaging suspicion of advanced liver disease).

T2DM, NAFLD and cardiovascular disease (CVD)

Whether the presence of NAFLD confers an increased risk of CVD beyond the presence of traditional risk factors is an area gathering substantial attention. Studies have reported that patients with elevated liver aminotransferases have a much higher Framingham risk score and/or cardiovascular events compared to those with normal liver aminotransferases [42, 64-66]. The association of NAFLD with the MetS has led to the connection between fatty liver and the development of CVD. The studies linking CVD with NAFLD can be divided into three major categories:

◆ small studies suggesting CVD occurs more commonly in the presence of NASH than in the general population or subjects without NAFLD [7, 12, 28, 29, 41, 66, 67];

◆ cross-sectional studies with surrogate measures of atherosclerosis in patients with NAFLD compared to controls; and
◆ longitudinal studies of cardiovascular events in patients with and without NAFLD at baseline (Table 3).

Table 3. Studies assessing cardiovascular burden in patients with and without NAFLD.					
Author (year)	(n) NAFLD vs. controls	Diagnosis of NAFLD by	Primary endpoint	Increased CVD	Adjusted CV risk #
Villanova et al (2005)	52 vs. 28	Liver biopsy	Endothelial function*	Yes	Yes
Brea et al (2005)	40 vs. 40	US	CIMT	Yes	No
Volzke et al (2005)	2961 vs. 1261	US	CIMT	Yes	No
Targher et al (2006)	85 vs. 160	Liver biopsy	CIMT	Yes	Yes
McKimmie et al (2008)	192 vs. 431‡	CT	CIMT	No	No
Fracanzani et al (2008)	150 vs. 250	US**	CIMT	Yes	Yes
Poanta et al (2011)	38 vs. 18‡	US	CIMT	No	No
Mirbagheri et al (2007)	156 vs.15	US	Coronary angiography	Yes	Yes
Targher et al (2005)	248 vs. 496‡	US	CV events	Yes	Yes
Hamaguchi et al (2007)	231 vs. 990	US	CV events	Yes	Yes
Schindhelm et al (2007)	888 vs. 551	ALT	CV events	Yes	Yes
Goessling et al (2008)	2517 vs. 295	AST, ALT	CV events	Yes	No

US = ultrasound; CT = computed tomography; CIMT = carotid artery intima-media thickness; ALT = alanine aminotransferase; AST= aspartate aminotransferase
# Cardiovascular (CV) risk after adjusted for traditional risk factors (age, gender, BMI, T2DM, MetS, other)
* Measured by brachial artery flow-mediated vasodilation
‡ All patients had T2DM
** 54 patients had liver biopsy

The major weaknesses of these studies are the diagnosis of fatty liver by ultrasound, a rather insensitive test with many false positives and false negatives, and the fact that in most studies patients with NAFLD typically are more obese, have more often diabetes and overall have a worse CV risk profile compared to the controls. These differences may or may not be able to be fully accounted for by multiple regression (or other) statistical analysis. In any case, as highlighted in Table 3, several studies have found that the positive association with CVD disappears after adjusting for traditional risk factors.

As mentioned in the section on natural history of NAFLD, series have been small and in general did not include proper controls. However, the available literature suggests that NAFLD carries a higher risk of CVD. For instance, Matteoni et al [29] was among the first to report increased CV events in a group of 132 biopsy-proven NAFLD patients followed for up to 18 years. Coronary artery disease was the second cause of mortality after neoplasm, although there were few CV events. Others have arrived at similar findings [28, 41, 67, 68]. Adams et al [67] followed 420 patients with NAFLD from Olmsted County, Minnesota, USA, between 1980 and 2000. Mean follow-up was 7.6 years and 12.6% died. Survival in the NAFLD population was significantly lower compared to the general population and liver-related mortality was higher than in the general population (hazard ratio of 1.34; 95% CI, 1.003-1.76; p=0.03), but again, both malignancies and coronary heart disease (CHD) were the leading causes of death. Recent reports appear to coincide with the observation that NAFLD increases CVD several-fold (ranging from ~two- to eight-fold) in this population [41, 68].

As can be appreciated in Table 3, previous studies have suggested a higher prevalence of coronary, cerebrovascular and/or peripheral vascular disease among patients with NAFLD compared with those without NAFLD when cardiovascular burden is measured by different methods (i.e. endothelial dysfunction, carotid artery intima-media thickness [CIMT], angiography, other). A study by Villanova et al [69] demonstrated an association between fatty liver and endothelial dysfunction measured by brachial artery flow-mediated vasodilation, a marker of early atherosclerosis, in 52 patients with NAFLD (39 with biopsy-proven NASH) compared to patients without NAFLD. Patients with NASH also had a higher 10-year probability of CVD events based on the Framingham risk score. Other studies have also used CIMT as a marker of atherosclerotic burden (Table 3), reporting that CIMT is markedly increased in patients with NAFLD compared to control subjects [70-74]. However, the association of NAFLD with CVD did not hold in several studies when adjusted for traditional risk factors of CVD [71, 72, 75, 76] and relatively few studies have reported that the association of NAFLD with CVD persists (i.e. CIMT and plaque prevalence) independently of the presence of components of the MetS [69, 73, 74, 77]. For instance, in patients with T2DM, McKimmie et al [75] and Poanta et al [76] reported that hepatic steatosis was not correlated with CIMT.

Studies that have examined the risk of CV events in patients with NAFLD have reported in general a higher risk of all-cause morbidity and mortality related to CVD than the general

population (Table 3) [42, 65, 69, 71-79]. As discussed earlier, a number of reasons may account for this [50, 80]:

- ◆ patients with NAFLD are often more insulin-resistant;
- ◆ frequently have dyslipidemia with elevated plasma triglycerides and low HDL-C;
- ◆ have worse subclinical inflammation; and
- ◆ be directly affected by FFA-induced endothelial dysfunction or myocardial lipotoxicity (Figure 2).

One study reported that among 2103 patients with T2DM, 5-year non-fatal CHD, ischemic stroke and CV death occurred more frequently in patients with NAFLD than those without a fatty liver measured by ultrasound [81]. Adjustment for traditional CV risk factors attenuated but did not completely abolish the association. Several studies used plasma aminotransferases
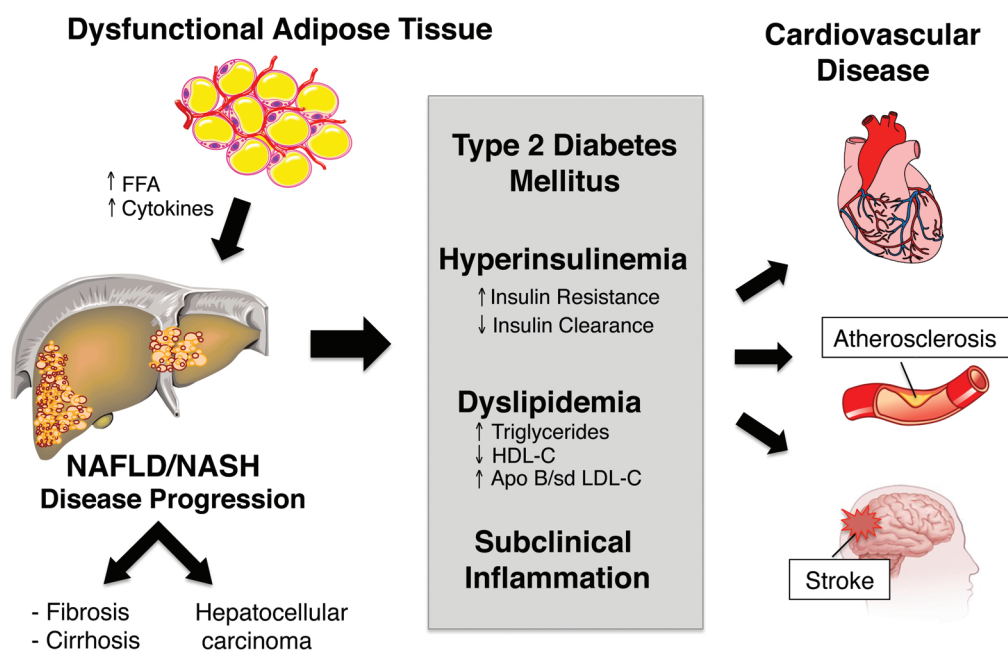

Figure 2. Dysfunctional adipose tissue leads to excessive free fatty acids (FFA) in the liver. This promotes triglyceride accumulation, hepatocyte lipotoxicity with necrosis, inflammation, and eventually disease progression to fibrosis, cirrhosis or hepatocellular carcinoma. The metabolic consequences are hyperglycemia, hyperinsulinemia, dyslipidemia and subclinical inflammation, all leading to premature cardiovascular disease. NAFLD: nonalcoholic fatty liver disease, NASH: nonalcoholic steatohepatitis, HDL-C: high-density lipoprotein cholesterol, sd LDL-C: small dense low-density lipoprotein cholesterol.

(ALT) as a surrogate marker of NAFLD [42, 64-66]. Ioannou *et al* [64] in a cross-sectional analysis found a strong correlation between elevated ALT and 10-year risk of CHD estimated by the Framingham risk score with a threshold for increased CV risk with an ALT greater than 43IU/L in men and 30IU/L in women. In 1439 patients from the Hoorn Study [65], the predictive value of elevated ALT for CHD persisted independent of the presence of MetS or other traditional risk factors. In the Framingham Offspring Heart Study [42], the development of MetS and diabetes was closely related to the presence of elevated ALT over a follow-up period of 20 years. However, while CVD was increased in age/gender-adjusted models in patients with NAFLD, this association was no longer significant in multivariate-adjusted models that included classical CV risk factors.

Thus, although metabolic and clinical factors would support the notion that NAFLD carries an increased risk of CVD, the evidence is inconclusive. Long-term prospective studies are needed to fully establish the nature of this association, but in the meantime, considering the many plausible pathogenic links between NAFLD and atherogenesis in T2DM, awareness and aggressive treatment of CV risk factors appears warranted **(III/B)**.

NAFLD: whom to screen and how to treat?

Diagnosis and screening

NAFLD is most commonly diagnosed by a combination of clinical, laboratory and imaging studies. A good medical history is important to rule out other causes of liver steatosis and it is essential to exclude significant alcohol intake. While the exact definition of excessive alcohol consumption in patients with NAFLD is unclear, alcohol consumption should ideally not exceed 15 grams per day (>12oz of beer, >5oz of wine, or >1.5 of distilled spirits). Accepted cut-offs are best tailored by gender with significant alcohol consumption defined usually as >21 drinks per week in men and >14 drinks per week in women over a 2-year period prior to baseline liver histology [82]. It is also recommended that a standardized questionnaire be used (i.e. AUDIT questionnaire).

Routine screening for NAFLD cannot be recommended at this time in all obese patients given our incomplete understanding about the natural history of the disease, factors leading to disease progression and treatment choices without definitive endpoints. At the present time, screening should focus only on those at the highest risk of having steatohepatitis (NASH) until a simple, non-invasive screening test with adequate sensitivity and specificity is available. These include patients with symptoms (i.e. right upper quadrant discomfort) or abnormal liver biochemistries (in particular, elevated aminotransferases). Patients in whom liver steatosis is an incidental finding detected on imaging, are asymptomatic and have normal plasma aminotransferase levels, a liver biopsy at the current time is not recommended [82]. However, as outlined earlier, plasma aminotransferase levels have a poor sensitivity as a screening test and are often within normal limits in NAFLD/NASH. While a liver ultrasound may assist in the diagnosis, it cannot be routinely recommended, given its cost and large number of patients with MetS or T2DM and NAFLD. In addition, its sensitivity is poor at low to moderate degrees of steatosis and it decreases further with increasing central adiposity.

At present, magnetic resonance imaging and spectroscopy (MRS) remains the gold standard for measuring liver triglyceride content but is available only in academic centers as it requires specialized software and interpretation is labor intensive. However, while it has a close correlation with liver fat content estimated from liver biopsies (Cusi *et al*, unpublished) it has a low sensitivity for necroinflammation and fibrosis.

Unfortunately, no imaging technique can replace a liver biopsy in confirming the diagnosis of NASH. A liver biopsy may be a reasonable approach to diagnose NASH and stage the disease (i.e. severity of fibrosis) in individuals who are at the highest risk of disease progression such as in obesity, MetS and/or T2DM, patients with symptoms and/or in the presence of elevated liver enzymes, and if the information obtained will prompt a more aggressive treatment approach. There are three general indications for liver biopsy:

◆   to confirm the diagnosis and establish the presence of NASH;
◆   to determine the prognosis based on the severity of liver fibrosis; and/or
◆   inclusion into a clinical trial in an academic center.

A liver biopsy should be considered only after an in-depth discussion with the patient about the benefits and risks of the procedure. In clinical practice it is done uncommonly given the high prevalence of NAFLD and the lack of specific long-term pharmacological therapies. This watchful waiting approach may soon change as new effective treatments emerge.

Finally, there is an active search for simple, reliable and inexpensive ways to screen the large number of obese and diabetic patients that are currently being missed with NAFLD or NASH. Efforts include the use of plasma biomarkers and novel imaging techniques such as transient elastography [80, 82]. While these approaches are promising they await more definitive validation before being recommended in the routine screening of NAFLD.

Treatment

Lifestyle intervention

It is well known that lifestyle interventions that result in weight loss, such as diet and exercise, decrease CVD and delay the development of T2DM [83]. However, the most effective lifestyle intervention in NAFLD remains unclear because there are no large, long-term trials. Weight loss clearly improves hepatic steatosis. Orlistat can facilitate weight loss but does not have an intrinsic effect on liver histology in NASH [84, 85]. A ≥7% weight loss with lifestyle intervention (200 minutes a week of moderate physical activity) can significantly improve liver steatosis, lobular inflammation and ballooning, but not fibrosis in obese patients with biopsy-proven NASH [86]. Reduction in steatosis ranging between 20% to 81% (measured by the gold-standard MRS technique) has been reported in multiple studies either by diet alone [87-90] or combined with exercise [88, 91-94], being proportional to the intensity of the lifestyle intervention. However, because a liver biopsy was not done in these studies, their impact on other aspects of liver histology remain to be established. The only study using both liver MRS and liver biopsies in patients with NASH reported that diet plus pioglitazone led to a 52% reduction in liver steatosis that correlated with a 50% mean decrease in steatosis, ballooning-necrosis

and inflammation on liver histology [95]. Roux-en-Y gastric bypass (RYGB) or laparoscopic adjustable gastric banding (LAGB) improve metabolic and histological abnormalities in NASH. However, changes in liver fibrosis have been much less predictable and some studies report that it may even worsen following bariatric surgery [96, 97]. Available bariatric surgery studies have been retrospective and poorly standardized in terms of pre- and post-surgical dietary procedures and lack of in-depth metabolic testing. There is an urgent need for a controlled, long-term bariatric surgery study to test the best approach for patients with NAFLD.

Pharmacological intervention

At the present time only vitamin E and pioglitazone have proven effective and safe for the treatment of NASH in short-term studies (6-24 months). How vitamin E benefits liver histology in patients with NASH is unknown but it appears to ameliorate oxidative stress [98-100]. However, its efficacy was conclusively demonstrated only in non-diabetic subjects with NASH [27].

Pioglitazone, an agonist of PPARγ most abundant in adipocytes [101, 102], improves adipose tissue/hepatic insulin action and improves liver histology in patients with NASH [2, 103]. In patients with prediabetes or T2DM and NASH, liver steatosis decreases by ~50% and necroinflammation improves in 85% of subjects [104]. These results were later confirmed in two randomized controlled trials in patients with NASH without diabetes [27, 105], although the results were less impressive perhaps due to the lower doses used (30mg per day versus 45mg per day by Belfort *et al* [104]). The PIVENS study [27] randomized 247 patients to vitamin E (800IU per day), pioglitazone 30mg/day, or placebo. The primary endpoint (histological improvement in ≥2 grades in the NAFLD Activity Score with at least a 1-point improvement in hepatocellular ballooning and 1-point in either the lobular inflammation or steatosis score, and no increase in the fibrosis score) was reached by 43%, 34% and 18% of patients treated with vitamin E, pioglitazone or placebo, respectively (significant at a p=0.04 versus placebo only for vitamin E). However, two factors affected the outcome of pioglitazone in the statistical analysis:

- patients that did not have ballooning at baseline, a component of the primary histological outcome, were considered as non-responders and more people in the pioglitazone versus vitamin E arm lacked ballooning at study entry (28% vs. 18%); and
- patients that did not have the second biopsy were also considered as non-responders.

Again, more patients in the pioglitazone arm did not have a second liver biopsy (13% versus 5% on vitamin E). Both therapies were equally effective in subjects with ballooning at baseline, the hallmark of NASH (pioglitazone: 52%, vitamin E: 47% and placebo: 23%, both significantly better vs. placebo). Both agents decreased hepatic steatosis (p=0.005 for vitamin E and p<0.001 for pioglitazone) and lobular inflammation (p=0.02 for vitamin E and p=0.004 for pioglitazone), but did not alter liver fibrosis. Resolution of NASH occurred in 36% of patients on vitamin E (p=0.05 vs. placebo), 47% on pioglitazone (p=0.001 vs. placebo) and 21% on placebo. Finally, it must be kept in mind that there is still concern about the long-term safety of pioglitazone regarding heart failure, bone loss and bladder cancer (European Medicines Agency, June 15, 2011, http://www.ema.europa.eu/ema) [106]. At the present moment, the FDA recommends avoidance of pioglitazone if a patient has active

bladder cancer, and caution if a patient has a prior history of the disease until more definitive data becomes available. However, cardiovascular disease has been reported to decrease with pioglitazone in patients with T2DM [80].

Other pharmacological agents used to treat T2DM have been tried in NASH such as metformin [107, 108] and incretin-mimetics such as exenatide [109, 110] or liraglutide [111]. Several uncontrolled trials with metformin [54, 103, 112-116] in patients with NAFLD have shown a reduction in plasma aminotransferases but the biguanide does not improve liver histology [103, 113, 115, 117]. The incretin-mimetics exenatide and liraglutide have generated significant interest because they induce weight loss and exendin-4 ameliorates hepatic insulin resistance and triglyceride accumulation *in vivo* [118]. Moreover, GLP-1 receptors are present in human hepatocytes [119, 120]. Exenatide has been reported to improve hepatic PPARγ/PPARα expression, Akt and AMPK phosphorylation in hepatocytes from patients with NASH [119]. While both agents exenatide [109, 110] and liraglutide [111] appear promising, randomized, controlled studies using liver histology as the main endpoint are needed before their use can be recommended for the treatment of NASH.

Conclusions

NAFLD in T2DM: distraction or impending disaster?

For decades, attention of the patient with T2DM has focused on the control of hyperglycemia and of risk factors associated with macrovascular disease. The epidemic of obesity now faces endocrinologists with new challenges. Among them is the need to identify early complications related to obesity in the setting of T2DM. In this sense, NAFLD is a common complication of patients with T2DM that requires special attention. However, it does not fit as such into the traditional realm of diabetic complications (i.e. micro- or macrovascular disease) but rather into a new genre of lipotoxicity-related complications in diabetes. Clinicians in general are uncomfortable with a condition that cannot be diagnosed or followed with a blood test, and that the natural history of the disease is poorly understood. However, the need for special attention about NAFLD in T2DM is based on some key findings:

- realization that the majority of patients with T2DM (>70%) have a fatty liver and a large proportion of these have NASH (≥50%?);
- severe steatohepatitis and disease progression occurs more often in T2DM;
- many mechanisms at play in NAFLD suggest that increased CVD is a real possibility, above and beyond traditional risk factors in patients with T2DM;
- pioglitazone remains as the most effective treatment for NASH in patients with T2DM [104].

While lifestyle intervention remains the cornerstone of treatment for NAFLD and the long-term safety of TZDs is under close scrutiny, pioglitazone will soon be generic in many countries. A diagnosis of NASH in a patient with T2DM may encourage its use at an earlier stage within the diabetes treatment algorithm to reverse the metabolic and liver-specific abnormalities of the disease.

In summary, although our current knowledge about NAFLD is incomplete and many aspects remain controversial, it is likely that this condition carries an inherent health risk to many patients with T2DM. Only our awareness and a proactive approach in the diagnosis and treatment of NAFLD in T2DM may avert an impending disaster looming on the horizon.

Acknowledgement

Key points	Evidence level
◆ NAFLD is the most common cause of chronic liver disease and as many as ≥40% of patients with NAFLD have steatohepatitis (NASH). NASH may progress to advanced fibrosis in a significant number of patients and is a major cause of cryptogenic cirrhosis.	IIb/B
◆ Obesity and diabetes (both related to dysfunctional adipose tissue and insulin resistance), or the presence of advanced hepatic fibrosis on a liver biopsy, are factors associated with NASH progression and poor long-term prognosis.	IIa/B
◆ Whether NAFLD is associated with an increased risk of premature death remains unknown. Evidence suggests that patients with NASH are at increased of liver-related morbidity and mortality, largely associated with the severity of liver fibrosis.	IIa/B
◆ The relationship between diabetes and NAFLD is complex. Obese patients with NAFLD are at increased risk of prediabetes (IFG, IGT) and of newly diagnosed T2DM compared to patients without NAFLD. Strong consideration should be given to screen patients with NAFLD for T2DM to establish an early diagnosis of diabetes and because patients with T2DM and NASH develop worse liver disease and have a two- to four-fold increase in advanced liver disease, cirrhosis and hepatocellular carcinoma.	IIa/B
◆ The mechanisms at play in patients with NAFLD support the notion that this condition carries an increased risk of CVD. However direct the evidence is weak and inconclusive. Long-term prospective studies are needed to fully establish the nature of this association, but in the meantime, aggressive treatment of CV risk factors appears warranted in this population.	III/B

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