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Time to replace assessment of liver histology with magnetic resonance-based imaging tests to assess efficacy of interventions for nonalcoholic fatty liver disease.

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Conflict of Interests: CDB is Principal Investigator for the WELCOME trial (**W**essex **E**valuation of fatty **L**iver and **C**ardiovascular markers in NAFLD (non alcoholic fatty liver disease) with **OM**acor thErapy), and the INSYTE trial (**IN**vestigation of **SY**nbiotic **T**reatm**E**nt in NAFLD). The WELCOME randomized placebo-controlled double blind trial tested the effects of high dose purified n-3 long chain fatty acids on a range of liver and cardio-metabolic outcomes in non alcoholic fatty liver disease (www.clinicaltrials.gov NCT00760513). The INSYTE randomized placebo-controlled double blind trial is testing the effects of a synbiotic on liver fat, disease biomarkers and intestinal microbiota in non-alcoholic fatty liver disease and is currently in recruitment phase (www.clinicaltrials.gov NCT01680640). Both the WELCOME and INSYTE studies assess quantitative changes in liver fat with the interventions by magnetic resonance spectroscopy, as one primary outcome in each trial.

GT has nothing to declare.

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Nonalcoholic fatty liver disease (NAFLD) is the most common metabolic liver disease worldwide and the burden of NAFLD is likely to increase with the epidemic of obesity and type 2 diabetes. The term 'NAFLD' embraces a spectrum of fat-related liver conditions, that begins with steatosis (nonalcoholic fatty liver or NAFL) and progresses to nonalcoholic steatohepatitis (NASH) with worsening fibrosis. Over time, liver fibrosis progresses, with the development of cirrhosis and increased risk of end stage liver disease. Current estimates of NAFLD prevalence are 20-30% for the overall adult population, and for patients with obesity or type 2 diabetes mellitus (T2DM), the prevalence of NAFLD is approximately 70-80% [1].

To date, there is no licensed treatment for NAFLD, which is often a 'silent' condition, characterized in its early stages by liver fat accumulation. Not only is NAFLD a risk factor for end-stage liver disease but, recently, it has become increasingly clear that NAFLD is also an emerging risk factor for many extra-hepatic diseases such as T2DM, cardiovascular disease (CVD) and chronic kidney disease [1].

Recent data from the United Network for Organ Sharing and Organ Procurement and Transplantation Network registry from 2004 through 2013 (on liver transplant waitlist registrants), showed that NAFLD is now the second most frequent indication for liver transplantation [2]. From 2004 through 2013, the greatest increase in patient numbers requiring transplantation occurred in patients with NASH compared with patients with other liver diseases. The authors concluded that for NASH, future research efforts should focus on preventing or delaying progression of liver disease, improving liver transplant waitlist survival, and improving liver transplant opportunities [2]. Since it has been projected that overall donor availability will significantly diminish in the next 15 to 20 years [2], it is urgent that new treatments for NAFLD are found, that are: effective, free from side effects, well tolerated and inexpensive.

In NAFLD, liver fat accumulation is the first stage of more serious chronic liver disease, and liver fat content exacerbates hepatic insulin resistance, predisposes to atherogenic dyslipidemia and also increases the risk of developing T2DM and CVD [1]. Moreover, not only is liver fat accumulation in NAFLD a risk factor for incident T2DM [3], but improvement/resolution of fatty liver appears to reduce risk of incident T2DM to the risk level of the population who never had fatty liver disease [4]. This finding has been further corroborated by the results of a recent prospective study showing that NAFLD improvement is closely associated with a reduced incidence of T2DM [5]. Recent discoveries of genetic variants [*e.g.*, patatin-like phospholipase domain-containing 3 (PNPLA3 148MM variant)], that influence NAFLD progression by influencing the hepatic lipid droplet, also adds credence to the importance of assessing liver fat accumulation *per se* in NAFLD. The PNPLA3 148MM variant impairs modification of the lipid droplet in hepatocytes, and this genetic variant plays a major role in the development and progression of NAFLD (triggering inflammation, fibrogenesis, and carcinogenesis) [6]. In addition, PNPLA3 148MM may not only modify liver lipid droplet accumulation, but may also influence the effect of potential treatments to decrease liver fat in NAFLD, such as high-dose long chain omega-3 polyunsaturated fatty acids [7,8].

NASH is thought to be a particular health concern due to the increased morbidity and mortality associated with progressive disease. To date, both the Food and Drug Administration (FDA) and the European Medicines Agency require the demonstration of improvements of liver histological endpoints for the market approval of any new pharmacological compound as a treatment for NASH. Specifically, it is believed that any new treatment for NAFLD should focus on improving NASH as the FDA does not recognise NAFL as a treatment indication. Since the focus therefore has been finding treatments for NASH (a condition that to date can only be diagnosed by histological examination of the liver), the FDA requires that new treatments for NASH should be proven to improve the histologically-derived NAFLD Activity Score (NAS) by two points with no deterioration in liver fibrosis.

The NAS score assigns a maximum of three points for steatosis, two for ballooning of hepatocytes, and three for inflammation. This biopsy-based approach to proving efficacy of a potential treatment for NASH has been predicated on the notion that improving histological features of NASH is key to reducing the risk of liver-related outcomes in patients with non-cirrhotic NASH. The reason for this approach has been largely based on the interpretation of data from retrospective cohorts of patients with NAFLD who have undergone an initial liver biopsy, showing that only NASH patients with increasing stages of liver fibrosis are at risk of progression to end-stage liver disease. Implicit in this approach is the notion that assessment of liver histology is being used as a surrogate for a clinically-relevant liver disease-related endpoint. We believe that such an approach has diminished dramatically a focus on finding treatments that decrease liver fat content *per se* as an early marker of disease, and thereby diminished attention on treating the liver condition in its early stages. Furthermore, since patients with NAFLD die two-fold more frequently due to CVD than to liver disease itself [1,6], it is also important to ensure that any new treatments for NAFLD do not cause harm beyond the liver. In particular, it is crucial that new treatments for liver disease in NAFLD do not increase the incidence of CVD and/or T2DM. Ideally, new treatments for NAFLD should not only benefit the liver, but also have a favourable impact on the risk of developing other NAFLD-related co-morbidities (such as CVD and T2DM). Although recent new treatments for NASH, such as obeticholic acid, have shown promise in some individuals with non-cirrhotic NASH [9], it is important to note that this bile acid-derived treatment was effective in 45% of patients, whereas placebo was effective in 21% of patients. Crucially, after 3 months of therapy, obeticholic acid caused a 75% increase in plasma low-density lipoprotein (LDL)-cholesterol concentrations [9]. For a liver condition that also increases risk of CVD, this increase in plasma LDL-cholesterol concentrations is a worrying side effect that may result in many patients requiring extra treatment with statins in order to treat this drug-induced increase in plasma LDL-cholesterol concentrations. Although obeticholic acid treatment for NASH is a potentially promising approach to ameliorate the liver disease, further long-term trials are needed with this agent to prove its long-term safety beyond the liver.

The prevalent belief that NAFL is harmless in NAFLD, is now being challenged. Increasing evidence is beginning to show that contrary to previous understanding of the pathogenesis of disease progression in NAFLD, there is a significant risk of developing substantial liver fibrosis over time, in patients who have simple steatosis (NAFL) as confirmed on initial liver biopsy. A recent important study from the Newcastle group in the UK [10] sheds further light on this issue. The Newcastle group evaluated 108 patients with NAFLD who underwent paired liver biopsies over a median interval of 6.6 years; 81 (75%) of these patients had NASH and 27 had simple steatosis at baseline. Overall, 45 (42%) patients had fibrosis progression, 43 (40%) had no change in fibrosis, while 20 (18%) had fibrosis regression. Importantly, no significant difference in the proportion exhibiting fibrosis progression was found between those with simple steatosis or NASH at first biopsy (37% *vs*. 43%, p=0.65). Notably, of 10 patients with simple steatosis, who had fibrosis progression, 3 progressed by 1 stage, 5 by 2 stages and 2 by 3 stages; and all patients had NASH on follow-up biopsy. Among the patients with simple steatosis, 80% of those having fibrosis progression had developed new T2DM at the follow-up liver biopsy, compared with 25% of non-progressors (p=0.005), further emphasising that developing T2DM is also a key risk factor for disease progression in NAFLD [10]. These results, albeit in a relatively small patient group, are not an isolated finding and support two previous smaller studies among NAFLD patients with two biopsies, performed more than one year apart, that showed similar results [11,12]. Although the findings of these studies need to be interpreted with some caution due to some intrinsic limitations (e.g., retrospective study design, small sample size, and lack of data on family history, genotypes, and body weight changes or medication use during the follow-up), they raise the concern that even patients with NAFL, who do not have NASH or advanced

fibrosis, are potentially at risk of progressive liver disease in the longer term. This conclusion has been further corroborated by the results of a recent meta-analysis looking at rates of fibrosis progression in 411 patients with NAFLD who had paired liver biopsies [13].

We believe that the results of these recent studies may have important clinical implications for newer and appropriate endpoints in future clinical trials designed to test the efficacy of interventions for NAFLD. Since the data discussed above[10-13] indicates that, contrary to popular belief, there is an important risk of progression with NAFL to more serious fibrotic liver disease over time, we suggest it is now an appropriate time to consider changing the focus of the primary endpoint used in therapeutic trials for NAFLD, be it pharmacologic, life-style [14] or surgical [15]. The aim of the primary care physician and the metabolic clinician at least, needs to focus on prevention or reversal of early disease. For the majority of patients with NAFLD, early disease is characterised by development of excess liver lipid (containing intra-hepatic triglyceride) and liver triglyceride can be easily and accurately quantified by magnetic resonance-based imaging techniques. Indeed, the quantification of liver triglyceride with these imaging techniques correlates very well with hepatic steatosis identified by histology [16]. In addition, these imaging techniques are more sensitive than the histology-determined steatosis grade in quantifying increases or decreases in the liver fat content and also provide better results than histology when steatosis has not involved the liver in a uniform manner [16,17]. Improving intra-hepatic triglyceride content assessed non-invasively by either the magnetic resonance spectroscopy-proton density fat fraction or the magnetic resonance imaging-proton density fat fraction [16,17], would allow a focus on the early stages of disease in NAFLD. Such an approach has been used recently by us[18] and by other investigators [19,20], and the use of magnetic resonance-based techniques would also consistently improve retention of participants within clinical trials. In our experience, many patients recruited to clinical trials that test interventions for NAFLD are reluctant to undergo potentially risky liver biopsies where there is no direct benefit for their own clinical care. In addition, the use of magnetic resonance-based imaging technologies in large, prospective observational studies would help also answer the question of whether we may improve the stratification of T2DM or CVD risk in patients with NAFLD, in order to therapeutically target the at risk individuals. A change of approach to using magnetic resonance-based approaches to testing primary outcomes in therapeutic trials for NAFLD would also save a considerable amount of money [~£700 *vs*. ~£300 (£1 = 1.53 USD 1 September 2015) for biopsy *vs*. magnetic resonance spectroscopy, in the UK at the present time]. Since improvements in NASH can only be quantified by liver histology, it is also difficult to assess patients during the follow-up period with repeat liver biopsies, negating the utility of the technique outside clinical trials.

Perhaps, it is now time to learn from our colleagues working in the field of CVD? In 1994, it was shown that long-term treatment with simvastatin significantly decreased future CVD events in people with established CVD (secondary prevention) [21]. For approximately a decade, statins were only used for the secondary prevention of CVD, with a focus on treating LDL-cholesterol concentrations to a specific target (so called 'treatment to target') in patients with established CVD. However, new guidelines from the American College of Cardiology and the American Heart Association represent a shift in our understanding of the benefits of statin treatment in people with 'normal' LDL-cholesterol concentrations at very modest cardiovascular risk who do not have CVD. These new guidelines now advocate treating people with statins at much lower CVD risk than ever we would have contemplated at the end of the 20th century. Rather than attempting to dichotomize and distinguish between NAFL and NASH (à la primary and secondary prevention of CVD), in our opinion people with NAFL need to be also included in future clinical trials so that interventions are found that are shown to be effective early in the disease process in NAFLD. If treatments are found that are safe and (relatively) inexpensive then those treatments should be available to all, even if it means treating more people with NAFLD.

Although replacing assessment of liver histology in NAFLD with non-invasive imaging assessment, is replacing one surrogate marker of liver disease with another, we propose that it is now time to focus on treating liver lipid accumulation as an easily detectable *early* manifestation of disease in NAFLD. Such an approach would not only place the focus on removing a trigger for liver disease progression, but would also have a beneficial effect on also focussing attention on modifying a risk factor for the development of important extra-hepatic complications of NAFLD such as T2DM, and maybe also CVD.

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