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# *Non-invasive Quantification of Fatty Liver Disease*

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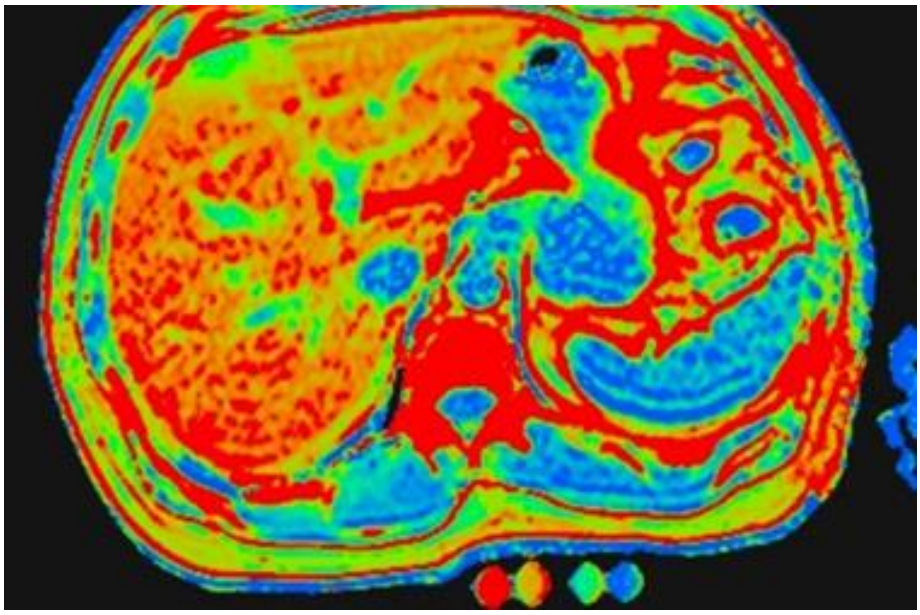

## Introduction

Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatisis (NASH) are emerging as the most prevalent causes of liver disease in Western countries. It is estimated that nearly 6 million Americans have NASH and nearly 600,000 have NASH-related cirrhosis [1].

As suggested by its name, NAFLD is caused by the accumulation of fat in liver cells (Figure 1). Clinically, the abnormal retention of fat in liver cells is termed steatosis. NASH, an advanced form of NAFLD, occurs when the liver becomes inflamed and damaged secondary to fat buildup. The damage caused by NASH is similar to that caused by long-term, heavy alcohol consumption, yet occurs in people who do not abuse alcohol [2]. The progression of NAFLD into NASH dramatically increases the risk of developing liver cancer and cirrhosis [3] – a condition where fibrotic "scar" tissue replaces healthy liver tissue, eventually interfering with the normal and life-sustaining functions of the liver.

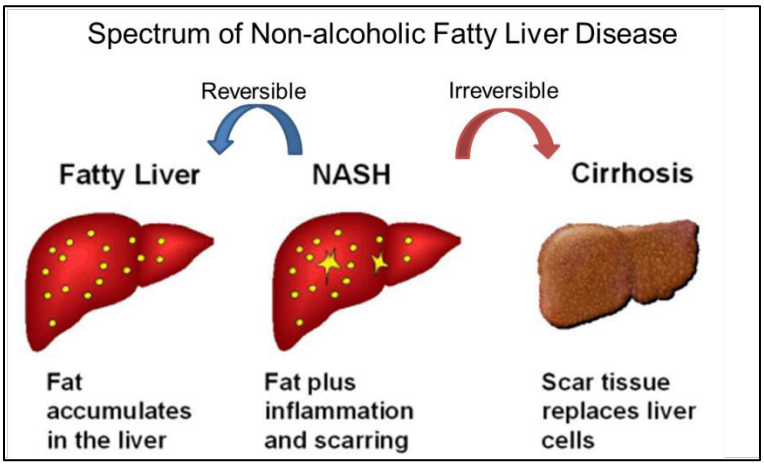


**Figure 1**. The progression of non-alcoholic fatty liver disease (NAFLD). Modified from [4].

## Symptoms and Disease Progression

NAFLD, and its progression into NASH, is considered a silent disease which occurs over years to decades with minimal symptoms. When present, symptoms include fatigue, weakness, weight loss, appetite suppression, nausea, jaundice, itching, edema, ascites, abdominal pain, and mental confusion [1,2]. Symptoms do not typically become apparent until the disease is well advanced or cirrhosis has begun. Since NAFLD/NASH is a slowly progressing disease, it is possible to slow, stop, or even reverse its effects on the liver (Figure 1). However, if NASH worsens, it can begin replacing healthy tissue with scar tissue, the precursor to cirrhosis. A person with cirrhosis can experience a range of symptoms, including fluid retention, muscle wasting, intestinal bleeding, and liver failure. Although reversible, NASH ranks as one of the major causes of cirrhosis in America.

## Causes of NAFLD and NASH

While the exact cause for NAFLD and NASH has not been elucidated, it is closely related to obesity, diabetes, insulin resistance, elevated cholesterol and triglycerides, hypertension, and rapid weight loss [2,3]. NASH is typically diagnosed in middle-aged persons but is also found in 3-10% of children [5]. The association of NASH with obesity and diabetes is staggering, with up two-thirds of diabetic and obese patients suspected of having NASH [5]. The rise of NASH worldwide is not surprising given that the World Health Organization estimates that over 1.9 billion adults and 42 million children under the age of five were overweight or obese in 2014 and 2013, respectively. It is important to note that not all people with NASH suffer from one of the above associated diseases and in some circumstances the cause of NASH is unknown.

## Diagnosis

NAFLD/NASH may be suspected if a person has elevated aminotransferase levels during routine blood testing. Imaging tests, including ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) can be used to determine non-invasively if steatosis is indeed occurring in the liver. However, the gold standard for diagnosing NASH is through a liver biopsy in which a needle is used to pierce the liver and extract a sample of tissue. NAFLD is diagnosed when histological evaluation of the liver tissue (Figure 2) shows fat deposition in greater than 5% of liver cells and NASH is diagnosed when there is also inflammation and damage present [6].

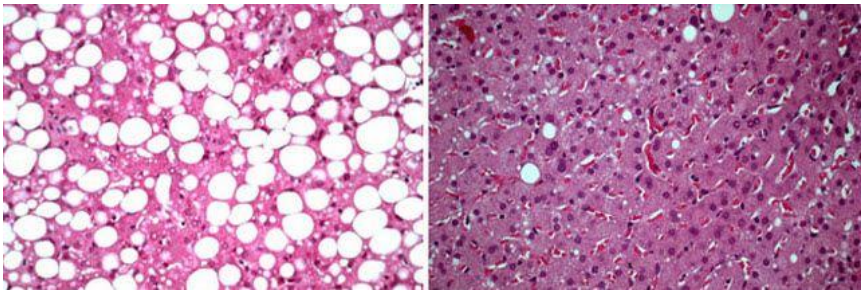

**Figure 2.** Histology sample showing a fatty liver (left panel; white globules) and the liver with improved histological features (right panel) [7].

## Treatment

Currently there are no approved drug therapies for NAFLD or NASH. Instead the diseases are managed through lifestyle changes aimed at eliminating the potential causes and risks factors. This may include weight loss, dietary changes, increased physical activity, discontinuation of drugs or toxins, reduced alcohol consumption, and treatment for high cholesterol or high blood sugar [2]. As noted by the World Health Organization, patients with a 5-10% reduction in weight show improved liver histology and enzymes [1].

## Evaluating Liver Fat

As mentioned above, biopsies are used routinely to evaluate fat content as well as other characteristics of the liver, including inflammation, damage and fibrosis. However, liver biopsies have a number of disadvantages. Specifically, biopsies are invasive, require hospitalization, can cause discomfort or bleeding, and in rare circumstances death [6]. Since the biopsy is only a small and random sampling of the liver, improper diagnosis and staging of the disease can occur if the acquired sample is not representative of the actual disease burden. It is has been estimated that sampling error may occur in up to 30% of liver biopsies [8]. Although biopsy is still the gold standard for diagnosing NAFLD and NASH, these limitations have served as the impetus for using non-invasive imaging modalities such as ultrasound, CT and MRI [9], as well as magnetic resonance (MR) spectroscopy to evaluate liver fat content. Medical imaging is used to identify the percentage of fat present in the liver, which is often referred to as the hepatic fat fraction.

### *Ultrasound*

Ultrasound is an appealing imaging technique for assessing liver fat content. Ultrasound is relatively simple, safe, painless, low-cost, and widely available. Indeed, several sonographic features have been shown to correlate with disease presence [10]. Recent studies have identified a relatively quantitative approach to using ultrasound to assess liver fat. Specifically,

the ratio of liver intensity to kidney intensity (termed the hepatic/renal ratio) has been shown to increase with increasing fat content and was found to correlate well with MR spectroscopy measurements [11]. However, ultrasound is operator-dependent, often causing variability in measurements between both imaging sessions and operators. Additionally, ultrasound is more qualitative than quantitative and it has poor sensitivity in detecting mild liver steatosis.

### *CT Imaging*

CT imaging can also be used to determine hepatic fat fraction and is done by evaluating changes in radiodensity. In CT imaging, the Hounsfield scale is used to quantitatively describe radiodensity (measured in Hounsfield units [HU]). The scale is defined by the radiodensity of water (0 HU) and air (-1000 HU). Comparatively, muscle is more radiodense than water (10 to 40 HU) while fat is less dense and thus has a negative radiodensity (-100 HU to -50 HU). As fat accumulates in the liver, the radiodensity of the liver decreases approximately 1.6 HU for each milligram of triglycerides per gram of liver tissue [12]. The hepatic fat fraction can be determined by comparing the liver radiodensity to a reference value. The reference value is frequently healthy liver tissue, which is between 50 to 57 HU. Alternatively, a patient's own spleen, which bears similar radiographic properties to the liver, can be used as the reference [13].

The use of CT is not without disadvantages. First, CT uses ionizing radiation which can have adverse effects on patient health. Exposure to ionizing radiation should always be "as low as reasonably achievable" (ALARA) which may limit the frequency at which imaging can be used to monitor liver fat. Secondly, as described above, assumptions of liver radiodensity are required when calculating fat fraction from CT images.

### *MR Spectroscopy*

MR spectroscopy, an analytical technique used to study metabolic changes in tissues, is the gold-standard for non-invasively determining liver fat measurements [14]. MR spectroscopy is conducted on the same machine as conventional MRI but requires ancillary software. While MRI uses hydrogen protons to form anatomical images, MR spectroscopy uses this information to produce a spectrum (i.e. graph; Figure 3) of the types and quantity of chemicals found in the tissue of interest. With regards to the liver, the spectrum is evaluated for peaks in fat and water and the fat fraction is determined by calculating the area under these peaks on the spectrum. Specifically, area under the fat peaks is divided by the sum of the areas under both the fat and water peaks [14].

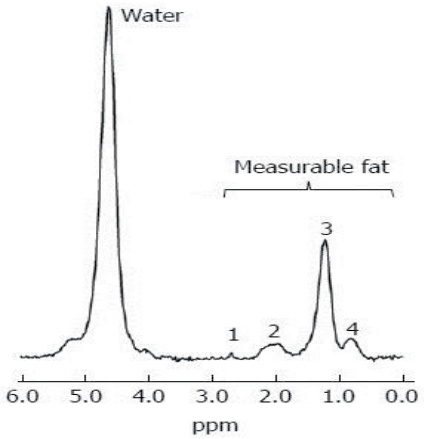


**Figure 3.** MR spectrum of the liver showing water and fat peaks [14].

Although highly accurate and sensitive for evaluating liver fat, MR spectroscopy is expensive and not as readily available as other imaging modalities, which limits its use in both clinical practice and large-scale clinical trials. Additionally, MR spectroscopy does not typically sample the entire liver. Instead, the operator specifies a sub-region of the liver from which the spectrum will be created. This can lead to a loss of spatial information and may make resampling of the same sub-region difficult at follow-up imaging sessions.

### *MR Imaging*

MRI can be used to quantitatively determine hepatic fat fraction. Liver fat is generally more difficult to quantify compared to other fat in the body (e.g. subcutaneous fat which is a layer of fat lying just under the skin). Liver fat is present at the sub-voxel level; therefore, it is not possible to simply count the number of voxels that are pure fat since each voxel contains some amount of fat and some amount of normal liver tissue. In order to calculate fat fraction, in-phase and out-of-phase images of the liver are used [15,16]. These images are created by taking advantage of the different spin rates of hydrogen atoms in water versus those in fat. For example, in a 1.5 Tesla magnet, hydrogen protons from water and fat become out-of-phase within 2.3 milliseconds, i.e., they point in opposite directions. After an additional 2.3 milliseconds the hydrogen protons are in-phase with one another [16]. The signal intensities from these two images are then used to calculate the voxel-level fat fraction [16,17].

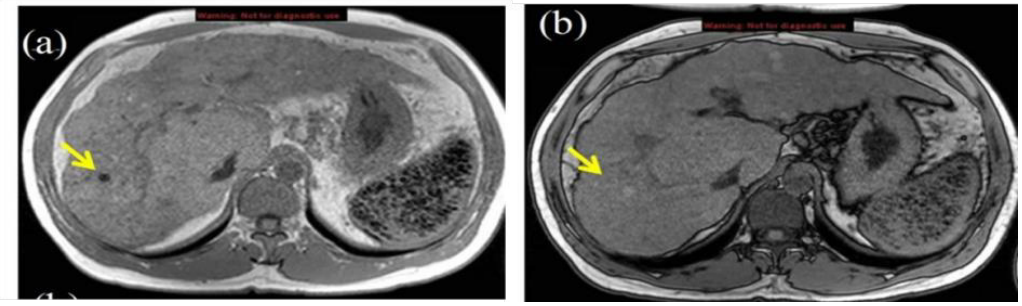


**Figure 5.** An in-phase image (left panel) and out-of-phase image (right panel) can be used to determine hepatic fat fraction [18].

There are different methods for calculating the hepatic fat fraction from in-phase and out-of-phase images. The simplest calculation is the two-point Dixon method which subtracts the signal intensity of the out-of-phase image from the in-phase image and divides this result by twice the in-phase image. Unlike other methods, two-point Dixon does not account for multiple types of fat that may be present in the liver nor does it correct for inhomogeneity in the magnetic field [16].

A more advanced approach is to use the three-point Dixon method which requires an additional in-phase image. T2*, which is a function of inhomogeneity in the magnetic field, is determined from the two in-phase images and is corrected for when calculating the fat fraction [16].

A third approach, the multi-interference method or proton density fat fraction (PDFF), is a very accurate method for calculating hepatic fat fraction. Specifically, this method is able to correct for T2* and model multiple fat moieties present in the liver [16,17,19]. The imaging protocol can be implemented on most MRI scanners but is a more complex acquisition requiring six in-phase and out-of-phase images. These three methods have been used to calculate hepatic fat fraction across multiple imaging sites with different scanner manufacturers and magnet strength [19]. Each is suitable for multicenter trials and longitudinal assessments; although, the multi-interference method is the most accurate of the three.

## BioTelemetry Research Provides Imaging Solutions for Fatty Liver Disease

BioTelemetry Research is a leader in hepatic fat imaging and quantification, providing unparalleled imaging solutions for phase 1 through phase 3 studies. Our operations team specializes in imaging risk identification and mitigation with a blend of flexibility across many therapeutic areas in clinical trials, which ensures seamless execution of liver imaging for your clinical study. Let our team work with you to determine the imaging modality, imaging acquisition protocol, and analysis method that will best serve your hepatic fat fraction study.

As with all studies, BioTelemetry Research is committed to providing quality data, on time, within budget, and on a consistent basis.

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