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Non-invasive, quantitative assessment of liver fat by MRI-PDFF as an endpoint in NASH trials

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is now the most common cause of chronic liver disease worldwide, and the progressive form of this condition, nonalcoholic steatohepatitis (NASH), has become one of the leading indications for liver transplant. Despite intensive investigations, there are currently no FDA approved therapies for treating NASH. A major barrier for drug development in NASH is that treatment response assessment continues to require liver biopsy, which is invasive and interpreted subjectively. Therefore, there is a major unmet need for developing non-invasive, objective and quantitative biomarkers for diagnosis and assessment of treatment response. Emerging data support the use of magnetic resonance imaging derived proton density fat fraction (MRI-PDFF) as a non-invasive, quantitative, and accurate measure of liver fat content to assess treatment response in early-phase of NASH trials. In this review, we will discuss the role and

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utility, including potential sample-size reduction, of using MRI-PDFF as a quantitative and non-invasive imaging-based biomarker in early-phase NASH trials.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is now the most common cause of chronic liver disease worldwide (1–3). NAFLD can be broadly classified into two categories: nonalcoholic fatty liver (NAFL), which is thought to have a minimal risk of progression to cirrhosis, and nonalcoholic steatohepatitis (NASH), the more progressive form of NAFLD, which is thought to have a significantly increased risk of progression to cirrhosis (4). Over the past two decades, NASH-related cirrhosis has become the second leading indication for liver transplants in the United States (5). For these reasons pharmacological therapy for NASH is urgently needed. Despite intensive investigations, there are currently no FDA approved therapies for treating NASH (6).

Need for non-invasive assessment in treatment trials

Currently, therapeutic trials in NASH require liver biopsy to establish an initial diagnosis of NASH and to document treatment response. However, it is an expensive and invasive procedure that carries potential risks (abdominal pain, bleeding, death) and is consequently disfavored by many providers and patients (7). Moreover, interpretation and scoring of biopsy are characterized by significant inter- and intra-observer variability (7, 8) and biopsy assesses only a small liver sample, approximately $1/50{,}000^{th}$ of the liver. Given the known spatial heterogeneity of diffuse liver disease, limited sampling of the liver can lead to meaningful errors in determining diagnosis, disease stage and longitudinal evolution (9, 10). These limitations directly impact clinical trials design as the diagnostic accuracy, reliability, and response to treatment end-points are key determinants of trial size requirements, feasibility, and costs. Furthermore, extending clinical trial findings to clinical routine practice remains a major obstacle due to barriers in obtaining repeated liver biopsies for treatment monitoring and patient follow-up. Therefore, non-invasive, reliable, accurate, safe and quantitative biomarkers are needed as an alternative to liver biopsy in clinical trials and to extend clinical trial practice to routine practice. Although histologic endpoint remain necessary in NASH clinical trials, emerging data support the use of magnetic resonance imaging derived proton-density-fat-fraction (MRI-PDFF) for treatment response assessment in early-phase trials in NASH for drugs which have an anti-steatotic mechanism of action.

MRI-PDFF

MRI-PDFF is a quantitative imaging biomarker that enables accurate, repeatable and reproducible quantitative assessment of liver fat over the entire liver (9, 11–14). Thus, MRI-PDFF is emerging as one of the leading non-invasive quantitative biomarkers suitable as a surrogate to liver biopsy for assessing treatment response in a subset of NASH trials.

In this review article, we will summarize the currently available evidence regarding the benefits of using MRI-PDFF in NASH patients and the advantages of this imaging technique compared to other methods especially when this imaging is used as an endpoint in NASH trials.

MRI-PDFF Methodology: How does MRI-PDFF quantify liver fat?

Chemical-Shift Encoded MRI and Proton Density Fat Fraction—MRI is sensitive to the signal from protons in mobile, unbound molecules such as water and triglycerides. Serendipitously, the signal from protons bound in structures such as the lipid bilayer of cells (including cholesterols, sphingolipids, and phospholipids) are invisible using conventional MRI.

Further, differential electronic shielding of protons in water and triglycerides leads to differences in the MR resonant frequency of different proton groups. This "chemical shift" between water and fat proton signals can be exploited by emerging chemical shift encoded MRI (CSE-MRI) methods to quantify the relative amount of water and fat signal arising from the tissue (15).

When all confounding factors such as T1 (16) and T2* (17–19), complex spectral characteristics of triglycerides (18–20), the noise behavior of MRI (16) and MRI system instabilities (21, 22) have been addressed, CSE-MRI methods can accurately measure the "proton density fat fraction" (PDFF) (23).

PDFF is defined as the ratio of the density of mobile protons from triglycerides and the total density of protons from mobile triglycerides and mobile water. It is expressed as an absolute percentage (%) and ranges from 0–100%. PDFF is a fundamental property of tissue that reflects the concentration of mobile triglycerides within that tissue. Although PDFF correlates closely with chemically determined tissue triglyceride concentration (24), PDFF and triglyceride concentration are not equivalent. Chemical-assay measurements of triglyceride include MR invisible chemical species that do not contribute to PDFF estimation. Similarly, PDFF is correlated with histological assessment of hepatic steatosis, which is expressed as the percentage of cells containing intracellular droplets of fat (25). Although closely correlated, these two metrics, both expressed as a percentage, are fundamentally different and not equivalent metrics of tissue fat content Figure 1.

Types of MRI-PDFF Strategies—Two primary CSE-MRI strategies have emerged, known as "complex" CSE-MRI and "magnitude" CSE-MRI. Complex-based methods utilize both the phase and the magnitude of the MRI signal and can fully separate the water and fat proton signals. The advantages of complex CSE-MRI include a full PDFF range from 0–100% fat concentration and improved signal to noise ratio (SNR) performance. Magnitude based methods utilize only the magnitude of the MRI signal, and thus are limited to a dynamic range of 0–50%, and lower SNR performance. However, magnitude based methods are less sensitive to system instabilities. Both methods provide highly accurate and precise estimates of liver fat concentration, which is almost always less than 50%, within the dynamic range of magnitude CSE-MRI. Both methods can rapidly assess PDFF over the entire liver in a short breath-hold (~20s). PDFF maps are automatically reconstructed without user input or post-processing – a major advantage over MR spectroscopy based methods Figure 2.

The data validating the use of advanced CSE-MRI methods to quantify PDFF is extensive. These include phantom studies (26, 27), animal studies (28, 29), ex vivo human liver tissue

(24), numerous studies comparing CSE-MRI to MRS (12–14, 30–43), as well as liver biopsy as the reference standard (44, 45). A comprehensive review of the technical details of CSE-MRI and data validating MRI-PDFF is beyond the scope and purpose of this review.

When to use MRI-PDFF as an endpoint in NASH trials?

MRI-PDFF is accurate, precise, and reliable, with excellent inter and intra-rater agreement (11, 32, 37, 46, 47). Furthermore, it should be noted that complex MRI methods have been successfully implemented, are FDA approved, and commercially available on the three major MRI vendors, GE Healthcare, Siemens and Philips, ensuring eventual wide-spread availability. In addition, it has been successfully applied in the setting of several clinical trials (32, 37, 48, 49).

Although MRI-PDFF is a useful tool, it is best suited for the following scenarios as a treatment endpoint in NASH trials.

1. When the drug or intervention has a high likelihood of an anti-steatotic effect.
2. Typically in an early phase trial to see if there is a significant reduction in liver fat content along with collateral improvement in an another biomarker (e.g. serum ALT or a plasma based biomarker) before moving on to a larger study using a biopsy-based endpoint.
3. Interventions that are associated with weight loss would also benefit in quantifying the relative reduction in liver fat content after intervention from baseline. This would help with sample-size assessment for a larger phase 2B or phase 3 trial with liver histology as an endpoint.
4. When the drug or intervention has a strong likelihood of pro-steatotic effect, it would be useful to include MRI-PDFF as an assessment of drug toxicity to quantify and assess the likelihood of harm to the liver (e.g. basal insulin have been shown to increase liver fat content)

The role and utility of MRI-PDFF can be illustrated using the example of a trial that was designed to assess the efficacy of colesevelam versus placebo in the treatment of NASH. In this trial, 50 biopsy-proven NASH patients were randomized to either colesevelam or placebo for 24 weeks (37). Based upon strong anti-steatotic from pre-clinical data in animal models, the primary hypothesis was that colesevelam would significantly reduce liver fat compared to placebo. Therefore, MRI-PDFF was an appropriate endpoint for assessment of treatment response in this clinical setting. Contrary to the study hypothesis, colesevelam was found to increase hepatic MRI-PDFF. This small but real increase in liver PDFF by colesevelam was only detectable with MRI-PDFF and not appreciable on liver histology assessment, due to the lower sensitivity of liver biopsy compared to CSE-MRI to detect small but real longitudinal changes. This example illustrates the utility of MRI-PDFF to detect small modification in liver fat content in the setting of clinical trial and further studies are needed to determine how well an improvement in hepatic steatosis correlates with resolution of NASH when a drug has different degree of anti-steatotic activity. It is also important to understand the challenges and limitations of using MRI-PDFF as a biomarker in early phase clinical trials.

1. When the drug or intervention has no or low likelihood of an anti-steatotic effect, MRI-PDFF is unlikely to be useful for the assessment of treatment response in NASH. Under such circumstances, alternative modalities and biomarkers should be considered.
2. Although MRI-PDFF is suitable as a steatosis marker, it does not assess NASH, fibrosis, inflammation, or other potential endpoints of interest
3. We anticipate that over the next several years, clinical trials will transition from magnitude-based to complex-based MRI-PDFF sequences. This will likely alleviate some but not all the quality control procedures currently needed. All of the commercially available MRI-PDFF methods are complex based, and as they are disseminated will become increasingly available.
4. MRI-PDFF may not be feasible in a small minority of patients including claustrophobic patients, patients too large to fit into the MRI scanner, and patients with contraindications to MRI such as certain metallic implants. MRI-PDFF does not require the administration of gadolinium based contrast agents.
5. MRI-PDFF sequences have relatively low accuracy for the detection of incidental but potentially important abnormalities such as liver tumors. Patients should be counseled that the MRI-PDFF is designed to measure liver fat, and that incidental abnormalities are not reliably excluded. However, it should be noted that it is very straightforward to include MRI-PDFF as part of a standard liver MRI protocol designed to detect and characterize liver tumors and other abnormalities.

Benefits of using MRI-PDFF

MRI-PDFF versus MRS-PDFF—Advanced MRS is considered the most accurate method for measuring PDFF. However, MRS has limited availability, is not fully supported by the system software on clinical MR scanners, usually needs to be run on special research modes, requires technical expertise for its acquisition and analysis, and only measures PDFF in one or a limited number of tissue voxels. The latter limitation introduces sampling variability, especially in longitudinal studies, because the voxel placement is difficult to replicate exactly with existing technology. Since MRI-PDFF and MRS-PDFF agree closely (13, 26, 32, 33, 37, 50), MRI-PDFF is generally preferred in clinical trials due to its greater practicality and lower sampling variability.

MRI-PDFF versus liver tissue—Historically, liver biopsy with histology scoring was the reference standard for hepatic steatosis. Due to its sampling variability and relatively broad grading categories, biopsy is insensitive to small but real changes in liver fat content. A major problem with using liver tissue as an endpoint in clinical trials is that true reductions (or progressions) in steatosis may be missed. Due to this problem as well as other limitations mentioned earlier, liver biopsy is not recommended as an endpoint in clinical trials if the primary outcome measure is steatosis reduction.

MRI-PDFF versus CT-attenuation; versus ultrasound-quantitative; versus ultrasound-qualitative; versus CAP—The attenuation X-rays passing through fat is lower than water, therefore livers with hepatic steatosis have lower attenuation on computed tomography (CT), an X-ray based imaging method (51, 52). Recently, Kramer et al. directly examined the relationship between PDFF and CT in patients undergoing same day CSE-MRI and non-contrast CT (53). Excellent linear correlation between PDFF and CT attenuation was observed (r^2=0.86), providing, for the first time, a direct calibration between PDFF and CT attenuation. However, while the correlation of PDFF and CT attenuation was strong overall, there was no meaningful correlation of PDFF and CT attenuation (r^2=0.04) at low levels of liver fat (PDFF < 6%). Thus, CT may be a useful biomarker to detect and quantify moderate to severe hepatic steatosis but its utility is limited, particularly at low fat concentrations that are likely the most clinically relevant (41, 54). Finally, the need for ionizing radiation makes CT less attractive, particularly in children, when alternative non-invasive imaging methods such as MRI are readily available.

Sonographic image brightness relates to the backscatter and attenuation of the ultrasound wave. Compared to lean liver tissue, fatty tissue scatters and attenuates sound waves. Hence, mildly fatty liver appears bright due to backscattered signals returning to the transducer. As the amount of liver fat increases, the ultrasound wave becomes attenuated. Radiologists assess these changes (brightening of the liver in the near field, darkening of the liver in the far field, blurring of vessels) qualitatively to determine the presence of steatosis. Although qualitative assessment for liver fat may be useful clinically, subjectivity and imprecision render this approach unsuitable for measuring steatosis and its longitudinal change in clinical trials. To provide an objective sonographic assessment of hepatic steatosis, new methods are being developed to quantify the degree of backscatter (55, 56) as potential biomarkers of hepatic steatosis. Among them, the controlled attenuation parameter (CAP), measured by Fibroscan (Echosens) allows a rapid assessment and is reasonably accurate for diagnosing the presence of steatosis (57, 58). However CAP is limited by high failure rates in obesity, lack of exact anatomic localization, and low accuracy for quantifying the amount of steatosis (59). The latter two factors make CAP unsuitable for use as an endpoint in clinical trials due to measurement imprecision and inability to monitor treatment response (60). Other quantitative ultrasound methods are investigational and not ready for use in clinical trials.

Correlation between change in steatosis vs change MRI-PDFF and between change in MRS-PDFF vs change in MRI-PDFF—Two recent studies, one in adults (32) and one in children (42) with known or suspected NAFLD, have shown that longitudinal change in MRI-PDFF agrees closely with longitudinal change in MRS-PDFF (with correlation coefficients of 0.96 to 0.99 and 0.986, respectively), when the MRI and MRS measurements at each time points are meticulously co-localized. Longitudinal change in MRI-PDFF after weight loss surgery is shown in Figure 3. These data provide further validation for the use of MRI-PDFF in longitudinal clinical studies that will help to determine the prognostic significance of the severity and change of steatosis.

How to use MRI-PDFF as an endpoint in NASH trials?

Site selection, qualification, training, and technical support—Although now commercialized by the three major vendors, complex-based MRI-PDFF sequences are generally available only on the latest-generation scanners. Even if available, the sequences may require the purchase of a license to enable their use. Therefore, a substantial proportion of sites participating in clinical trials currently or in the next few years are unlikely to have access to these advanced sequences. Until now, therefore, most clinical trials have used magnitude-based MRI-PDFF sequences. These usually can be implemented by using commercial sequences developed for other purposes and then modifying the acquisition parameters as needed, often under the guidance of a central radiology coordinating center.

Site selection for clinical trials is usually based on hepatology expertise and enrollment capacity, not imaging capability. Hence, the radiology coordinating center must determine the relevant technical capabilities of each site, including field strength, scanner manufacturer, and scanner software. Based on this information, a standardized protocol is developed that is both feasible at every site and adequate for reliable PDFF estimation. A detailed MRI procedure manual is given to the sites, which usually provides all the necessary training. If needed, additional questions can be answered by email or teleconference. Site qualification is done in parallel with training and begins with a review of the site's technical qualifications. The ability to perform the exact protocol is then confirmed by scanning a phantom (an inanimate object such as a bottle of water) or a human volunteer. Images are sent to coordinating center, which verifies that all parameters are within the allowable range. Additionally, the coordinating center provides as-needed technical support if sites are unable to acquire images using the allowed parameters or diverge from the protocol at any point in the study.

Quality control: acquisition, intake, analysis, reporting—Quality control (QC) is essential. The technologist performing the study at each site is responsible for verifying MRI safety for each patient, positioning the patient correctly, adhering to the research protocol, checking images as they are collected, and repeating any images that are degraded by a correctable error such as sudden motion from a cough. The coordinating center is responsible for intake QC (verifying all parameters are within range, the appropriate anatomy was covered, images are of adequate quality for analysis), analysis QC (verifying that the analysis described below follows a standard operating procedure, that the exact locations of the regions of interest are recorded, and that the values taken from the images are within expected range or rechecked), and reporting QC (ensuring that the values recorded form the images match the values inputted into the database and that the final imaging database is complete)

Analysis: co-localization, number of ROIs, size of ROIs—Once PDFF maps have been acquired, analysis of these maps must be performed to derive a single PDFF estimate for that MRI exam. There are many possible approaches and currently there is no official consensus on how to analyze PDFF maps. Region of interest (ROI)-based methods that measure the average PDFF value in a region of the liver are generally used, and standardized approaches are emerging. Standardized approaches that have excellent intra- and inter-reader

variability are preferred, although they may be labor intensive. Whole-liver segmentation that avoids large blood vessels, bile ducts, liver lesions and image artifacts would likely provide the best intra- and inter-reader variability, but are not practical and probably not necessary. The use of multiple large ROI's is usually sufficient to provide adequate sampling of the liver. Hines et al first proposed a sampling strategy that placed one ROI per Couinaud segment (61), while other groups have described the use of four ROI paradigms, with one ROI in the anterior, posterior, medial and lateral segments (62). More recently, Campo et al rigorously evaluated the effects of the number of ROI's used, ROI size, and ROI location on the intra- and inter-reader variability of PDFF measurements, and evaluated the time burden required for different ROI strategies. Based on the results of this study, we conclude that the use of large ROI's ($\geq 4 cm^2$) with either a 4-ROI paradigm (anterior, posterior, medial, and lateral) or 9-ROI paradigm (Couinaud segments) are preferred to provide the best intra- and inter-reader variability with an analysis time of approximately 1–2.5 minutes by an experienced user (63)

Longitudinal changes: what is the quantitative change in liver fat that is associated with improvement in liver histology?—In order for MRI-PDFF to be more widely acceptable for the assessment of treatment response in NASH, one of the key data that are needed are the amount of liver fat decline that is clinical meaningful. Long-term studies are needed to assess whether a sustained and significant reduction in liver fat will lead to improvement in fibrosis, reduction in the risk to progression to cirrhosis and reduction in the risk of death from liver disease. This may be a task that is not likely to be achieved in the near future. Therefore, investigators have initiated seminal studies that aim to solve this puzzle by answering questions that can be answered in the near future. One such attempt was done by Patel et al using paired MRI-PDFF and liver histology data from two high quality randomized trials. Utilizing paired MRI-PDFF and liver histology data, Patel *et al.* demonstrated that a relative reduction of 29% in liver fat on MRI-PDFF is associated with a histologic response in NASH (defined as a 2-point improvement in NAFLD activity Score) (64). Although these preliminary data need to be confirmed in larger cohort, they are now being used to design future NASH clinical trials using the change in hepatic fat quantified by MRI-PDFF as a treatment endpoint. Future studies are needed to assess whether there is a tipping point for liver fat reduction that if sustained over a 6 month period would be associated with either resolution of NASH or improvement in one stage of fibrosis.

CONCLUSIONS

Non-invasive, quantitative, precise and reproducible, MRI-PDFF is emerging as a useful biomarker to assess treatment response in the setting of early phase clinical trials in NASH. It is suitable for quantifying liver fat content, however, it does not assess NASH, fibrosis, inflammation, or other potential endpoints of interest. Therefore, multi-modality assessment of treatment response is needed to examine the treatment response as MRI-PDFF is restricted to the liver fat domain alone. However, if there is a large enough quantitative decline in liver fat content it may also be associated with other collateral benefits such as improvement in inflammation. Emerging data suggests that a relative decline of 30% or more may be clinically meaningful but remains to be validated in larger studies. We

anticipate that over the next several years, clinical trials will transition from magnitude-based to complex-based MRI-PDFF sequences. This will likely alleviate some but not all the quality control procedures that are currently needed, and help standardize liver fat quantification. Further research is being conducted to develop an MRI-based package including MRI-PDFF, two-dimensional magnetic resonance elastography (2D MRE) and three-dimensional (3D) MRE and other MR based biomarkers to have a comprehensive liver disease assessment. The future is extremely bright for non-invasive assessment of treatment response using imaging modalities with an exponential increase in innovative applications of MR-based modalities. Such novel quantitative imaging modalities will likely continue to transform clinical trial design in the years to come.

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Abbreviations

AUROC	area under the receiver operator characteristic curve
BMI	body mass index
CAP	controlled attenuation parameter
CI	confidence interval
CSE-MRI	chemical shift encoded magnetic resonance imaging
CT	computerized tomography
DECT	dual-energy computerized tomography
FDA	U.S Food and Drug administration
HCC	hepatocellular carcinoma
MRI	magnetic resonance imaging
MRI-PDFF	magnetic resonance imaging proton density fat fraction
MRS	magnetic resonance spectroscopy
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
TE	transient elastography
VCTE	vibration-controlled transient elastography

ROI region of interest

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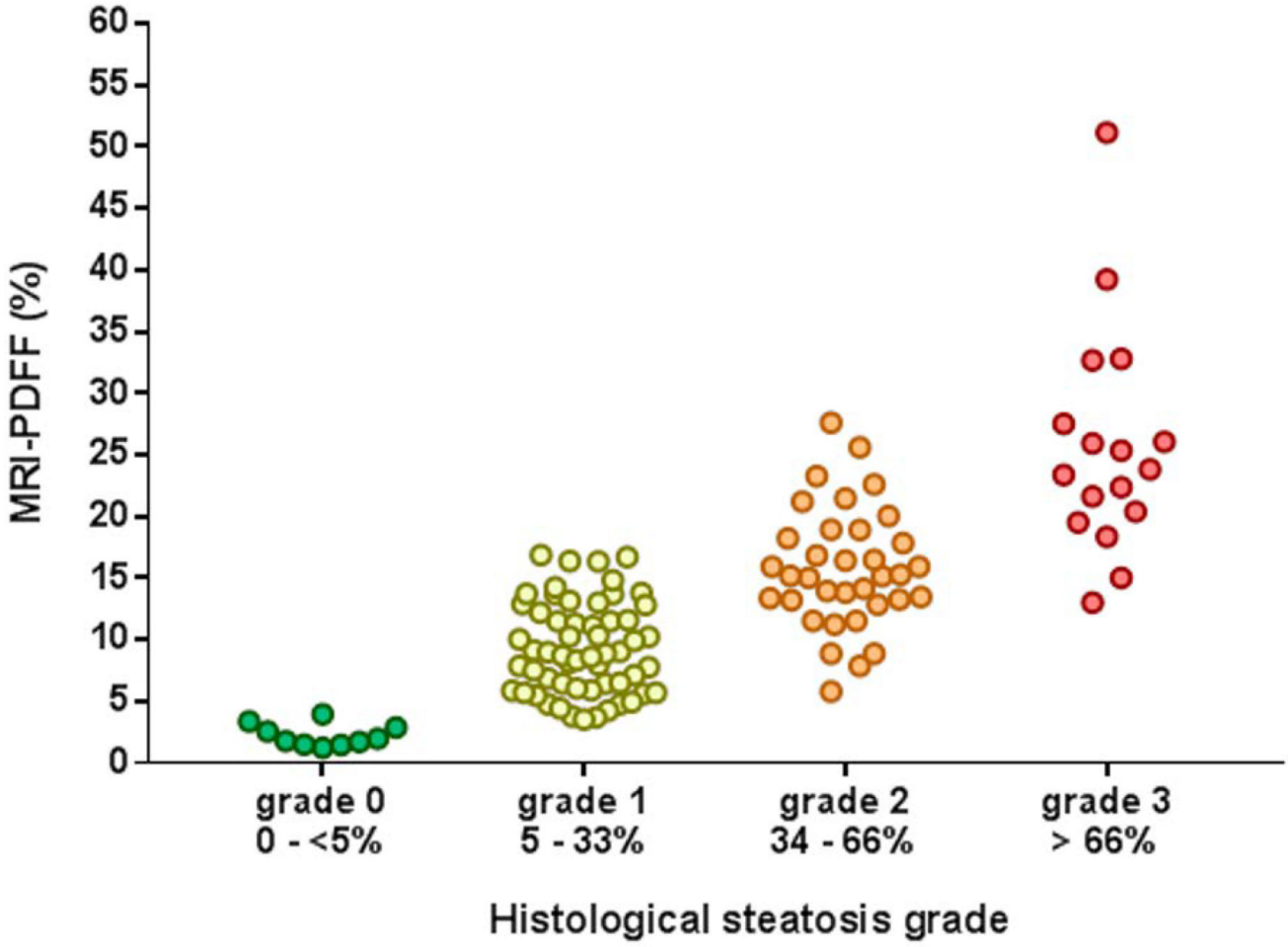

Figure 1. Correlation between MRI-PDFF and percentage of hepatocytes with steatosis by histology

Correlation between MRI-PDFF and histologic steatosis grade classified by the percentage of hepatocyte with steatosis using (25) in individuals with biopsy-proven NAFLD (60).

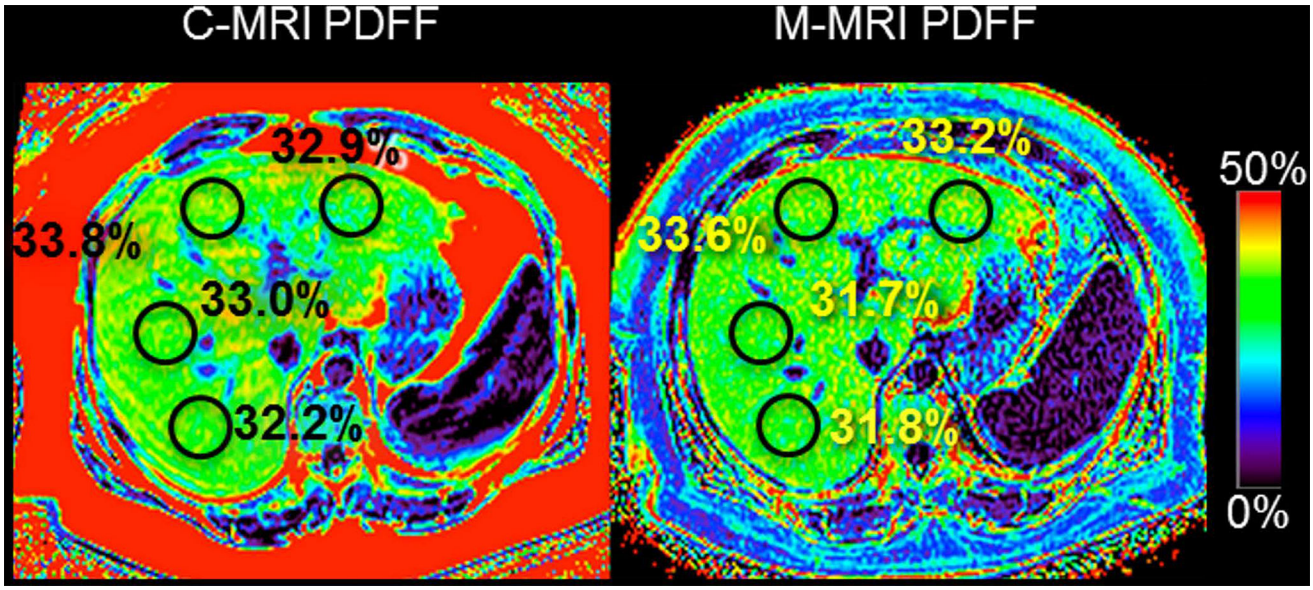

Figure 2. MRI-PDFF assessment and quantification of hepatic steatosis

Example PDFF maps using complex MRI (C-MRI, left) and magnitude MRI (M-MRI, right) both show elevated PDFF in the liver (~32%). M-MRI is limited to a dynamic range of 0–50% unlike C-MRI which has a full dynamic range from 0–100%.

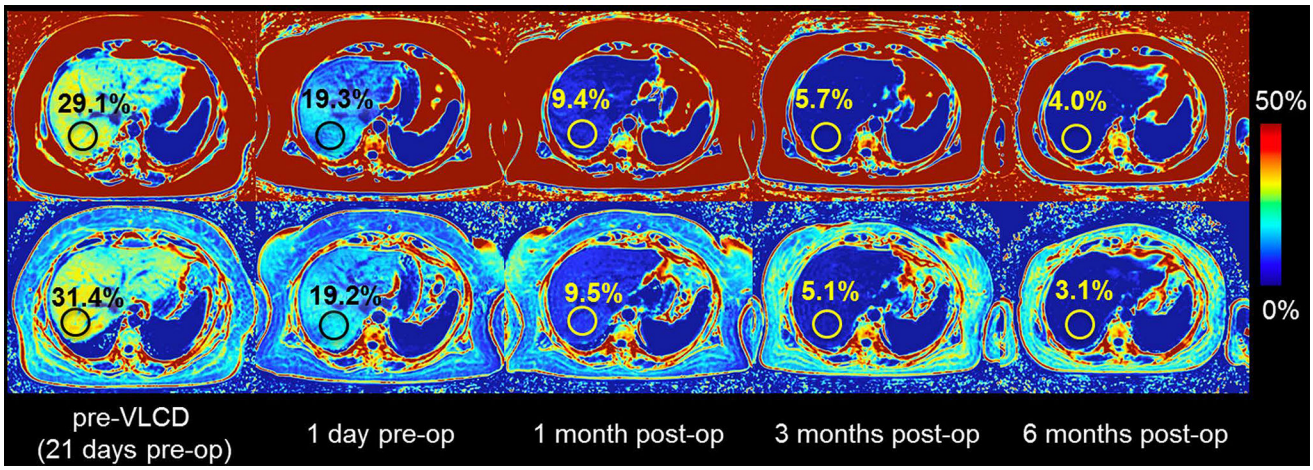

Figure 3. Longitudinal changes in MRI-PDFF after weight loss surgery

PDFF is shown in a patient before (pre-op) and after (post-op) weight loss surgery. Images from left to right represent PDFF before a very low caloric diet (pre-VLCD) pre-op and the longitudinal follow-up showing a decrease in PDFF.