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MRI overview for fat quantification in non-alcoholic fatty liver disease in the clinical and research settings

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Thesis

**MRI OVERVIEW FOR FAT QUANTIFICATION IN NON ALCOHOLIC FATTY
LIVER DISEASE IN THE CLINICAL AND RESEARCH SETTINGS**

by

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B.A., Columbia University, 2011

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ABSTRACT

The general purpose of this master's thesis is to describe the MRI techniques used in scanning and post processing for quantifying liver fat percentages for the purpose of diagnosis and research. At the onset we will look at epidemiological data regarding nonalcoholic fatty liver disease, which is often called by the name of hepatic steatosis. Based on the prevalence of this disease it is worthwhile to fully understand non-invasive (MRI) analysis, and its use in the clinical and research setting. Following an introductory section regarding the basis of magnetic resonance imaging, we will take a more in-depth look at current methods utilized for liver fat quantification. Due to the massive population of those of suffer from this disease worldwide it is prudent to analyze current methods, as well as the implications that such research has and will have on the pharmaceutical approach to treating this disease. The purpose of this thesis is to elucidate the MRI techniques utilized for liver fat quantification and provide a comprehensive view of how these techniques are used for diagnosis in the clinical setting, and longitudinal studies in the research setting to measure liver fat levels and how they react to various treatment approaches.

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LIST OF ABBREVIATIONS

DM2	Diabetes Mellitus Type 2
HCC	Hepatocellular Carcinoma
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NAFLD	Non Alcoholic Fatty Liver Disease
NASH	Non Alcoholic Steatohepatitis

INTRODUCTION

Non-alcoholic fatty liver disease in today's world is an extremely prevalent cause of liver disorder (Alfire et al). Thirty per cent of the total population of the United States has hepatic steatosis. Of this cohort, ten to twenty percent have steatohepatitis, cirrhosis or fibrosis (Browning et al). These numbers increase significantly when we look at obese individuals. Within this group, seventy five percent have steatosis and twenty to thirty-five percent have nonalcoholic steatohepatitis (commonly referred to by the acronym NASH). About half of patients with type two diabetes also have NAFLD and, not taking in to account body mass index, have worse disease of the liver than non-diabetic patients. NASH is also closely related to both elevated occurrence rates of liver-related mortality and cardiovascular disease.

The impact of nonalcoholic fatty liver disease on health care resources both in the US and around the world is significant. Adults with this disorder have been reported to incur twenty six percent greater total health care costs (Baumeister et al). There is convincing evidence for increased cardiovascular disease resulting in death, especially in patients with DM2. However taking into account the current widespread nature of obesity and DM2, the drain of nonalcoholic fatty liver disease on global health care will continue to increase noticeably in coming years.

Augmented appreciation of the significance of NAFLD in recent years has caused the inception of a multitude of studies regarding several differing treatment modalities. These methods have included lifestyle revision, pharmacological

treatment and intervention via surgery. The main objective of any NAFLD therapeutic technique is enhancement in treatment of fibrosis and steatohepatitis, with the eventual goal of preventing cardiovascular disease and liver related death.

NAFLD is one of the most prevalent underlying causes of reoccurring disease of the liver in the United States. Noninvasive recognition and quantification of fat is becoming increasingly important clinically because of the growing population of those who suffer from nonalcoholic fatty liver disease. Steatosis, which can be defined as the buildup of vacuoles which contain fat in hepatocytes, is a key histologic hallmark of fatty liver disease. However liver biopsy, which is the current commonly used measure in hospitals around the world for detection of steatosis has some drawbacks. Biopsy, for one is invasive, often has sampling errors, and is not always appropriate in some situations. Several magnetic resonance (MR) imaging methodologies, which include chemical shift imaging, MR spectroscopy, and frequency-selective imaging, are currently being utilized in the clinical setting for the quantification and detection of hepatic fat, with each technique having important positive and negative assets and liabilities. These techniques are able to quantify fat percentages by analyzing the differences of the net MR signal and its fat and water signal components, allowing for the measurement of fat in liver tissue, and are often being utilized in the treatment, diagnosis, and measurement of subjects with fatty liver disease.

INTRACELLULAR FAT: HEPATIC STEATOSIS

Steatosis is known to be a hallmark component of liver disease. Hepatic steatosis is described as the atypical and disproportionate intracellular buildup of fat in hepatocytes, primarily in the form of triglycerides. Long known to be an accompanying consequence of other indications such as diabetes or obesity, steatosis is now recognized as having an instrumental function in important hepatic and systemic disorders (Adams et al).

For example, non-alcoholic fatty liver disease is present in twenty to eighty million Americans and is one of the most common chronic liver diseases in the United States (Clark et al). Steatosis is the instigating process in NAFLD and can cause cirrhosis. Free fatty acids, which are the substrate for triglyceride creation, activate cell death by stimulating oxidative strain, inciting creation of reactive oxygen species and cytokines, and activating cell death, possibly causing progressive hepatic disorder. Studies have shown that five to fifteen percent of subjects with non-alcoholic fatty liver disease present with already occurring cirrhosis in liver biopsy and that four to five percent of subjects with isolated steatosis will often eventually have cirrhosis. Steatosis has also been known to worsen the course of viral liver disease. For example, in chronic hepatitis C infection, steatosis may diminish the effectiveness of antiviral therapy and hasten disease development. Furthermore, steatosis decreases hepatocellular functional reserve and contributes to postoperative hepatic failure after liver transplantation or resection.

Rising evidence indicates that hepatic steatosis increases risk of malignancy. The risk of hepatocellular carcinoma is particularly high; seven percent of patients with NAFLD-related cirrhosis developed hepatocellular carcinoma over a ten year timeframe (Sanyal et al). Because of the large prevalence of NAFLD in general population, it is possible that over fifty thousand Americans will ultimately develop NAFLD-related HCC. Worryingly, new reports describe HCC in patients with NAFLD without cirrhosis or fibrosis, suggesting that hepatic fat may have direct carcinogenic effects.

Hepatic steatosis may add to diabetes by interfering with insulin signaling and may be a pathogenic connection between obesity and its subsequent metabolic complications. In other studies, twenty to fifty percent of individuals with steatosis subsequently became diabetic. Furthermore, cardiovascular disease is one of the most common causes of morbidity and death in patients with NAFLD. This association has previously been credited to morbidities of NAFLD (obesity, hypertension, diabetes, and dyslipidemia) rather than NAFLD itself but new data show that NAFLD is as a risk factor for cardiovascular mortality. Of particular concern is that the increased cardiovascular mortality associated with NAFLD begins at age 45.

TREATMENT OF FATTY LIVER DISEASE

Liver fat is a meaningful marker of, and a contributor to, both hepatic and systemic morbidity and mortality. Hepatic steatosis is not a benign process and has important implications for many important diseases including cancer, diabetes and cardiovascular disease. Fortunately, steatosis can be changeable with involvement, and decrease in liver fat may lessen many of its associated risks.

Weight loss, by means of exercise and diet are vital to progress in obesity related liver fat disease although the exact nature of the relationship between weight loss and liver steatosis is poorly understood. Moreover, the majority of NAFLD patients are unsuccessful at attaining adequate weight loss and often other means are required. Laparoscopic gastric banding for example has been shown to be an effective surgical treatment of obesity and has been shown to reduce liver fat (Heath et al).

Exciting new pharmacological interventions of NAFLD that target its underlying insulin resistance have lately occurred. Lin et al demonstrated a marked decrease in steatosis in a leptin deficient mouse model of steatosis and insulin resistance, when treated with metformin, a member of the biguanide drug class, known to improve insulin sensitivity. Recently, Bugianesi et al have shown a significant decrease in hepatic steatosis in patients treated with metformin. In addition, it has been shown recently that NAFLD patients treated with pioglitazone display improved hepatic steatosis. Pioglitazone is a member of the thiazolidinedione drug class, and like metformin, improves insulin sensitivity.

Based on results such as these, we see that the NIDDK Clinical Research Network for NASH has two trials nearing their end for evaluating new treatments for NAFLD. The PIVENS30 trial will compare the efficacy of pioglitazone (vs placebo or vitamin E) in adults, while the TONIC31 trial will gage the effectiveness of metformin (vs vitamin E or placebo) in adolescents. Valuation of steatosis will rely on biopsy, which will be limited to the start and conclusion of these trials, due to the cost and risks related with biopsy. Exact quantification of steatosis with a magnetic resonance imaging based method in these studies would have allowed for frequent evaluation with a greatly improved safety profile and reduced expense, tracking the time course of steatosis during each trial. Such methods method could potentially transform the translation of new therapies from experimental drugs into clinical practice.

MRI OVERVIEW

The basis of Magnetic Resonance Imaging is the result of the magnetic properties of water and fat within the human body being utilized to make an image of a particular anatomy. This is done by utilizing the magnetic properties of the protons within the hydrogen nucleus which are inherently abundant within the body.

For a basic, or classical (vs. quantum mechanical) understanding of MRI principles, we can consider the hydrogen proton to be spinning on its axis with a north to south orientation. Due to the inherent electrical charge each proton possesses, the spinning of the proton results in a small magnetic field referred to as a proton magnetic moment. In resting state these spinning protons are randomly assorted on an infinite number of axes in the human body.

Once a subject is placed in the magnetic resonance imaging machine's extremely strong magnetic field (typically 1.5 or 3 Tesla), the net resultant alignment of all of the proton magnetic moments will be in one direction; the direction of the main scanner magnetic field. This resultant orientation of proton magnetic moments can be considered as a resultant vector with an orientation in the direction of the MRI magnetic field. This resultant vector is usually referred to as the net magnetization.

The next step in the process of creating an MR image involves deflecting the direction of the net magnetization, through the use of an electromagnetic field oriented in a direction perpendicular to the direction of the MRI scanner magnetic

field (B_0). The magnetic field component (B_1) of this electromagnetic pulse perturbs the net magnetization vector, albeit briefly, by rotating it 90 degrees from its original direction. To most efficiently ensure the rotation of the net magnetization, the B_1 field is designed to oscillate at a frequency in the range of radio waves, hence the term radiofrequency or RF pulse which ensures efficient transfer of energy to the net magnetization vector. This optimal frequency is referred to as the resonant frequency and is the impetus for the term “resonance” in magnetic resonance imaging. Practically speaking, matching the resonance frequency of the hydrogen nuclei is achieved by tuning the radiofrequency pulse to the frequency which will cause the hydrogen nuclei to resonate; this frequency is known as the Larmor frequency and is given by $\omega = \gamma B_0$ where gamma is a constant referred to as the gyromagnetic ratio, and B_0 is the magnetic field strength of the MRI scanner.

Next a change or gradient in the main MRI scanner magnetic field is created in the anatomical area of concern in order to spatially localize the net magnetization vectors (one net magnetization vector per volume element or voxel), by using three electrical coils which are wound around the bore of the MRI scanner, thus linearly changing the magnetic field throughout the anatomy of interest. This is how slices are created in an MR image.

Next the RF frequency is turned off so that the net magnetization in each voxel will go back to their resting state or state of “equilibrium”. As a result of this return to equilibrium, a signal is induced into a receiver coils which must also be

tuned to the Larmor frequency. The time varying and very small voltages induced in the receiver coil are transformed from the time “domain” to the frequency domain by a process referred to as the Fourier Transformation, which is necessary to provide a grey scale image on the scanner display console.

The intrinsic atomic makeup of the various tissues in the human body result in differing rates of relaxation when the RF pulse is switched off. This results in two predominant relaxation times which are referred to as T1 relaxation and T2 relaxation. T1 relaxation time is the result of the time necessary for the net magnetization to return to its equilibrium value following excitation by the RF pulse. The T2 relaxation time is the time necessary for the individual spinning protons which compromise each net magnetization to go out of phase with each other in a plane perpendicular to the main axis of the MRI scanner.

The creation of pulse sequences is of crucial importance to the operation of the MR scanner and is the best way to elicit the most accurate results, and useful MR images. Common areas of study involve separating water and fat from each voxel, which require specific pulse sequences are used which vary depending on the intrinsic relaxation times involved. An example of this is a fat suppression pulse sequence, which works by removing the fat signal, leaving only a signal given off by the other tissues in the field of view.

Some draw backs of MR imaging in general are the fact that certain pathologies will be difficult to distinguish from one another, for example a tumor and an infection. This is when human analysis of images is necessary. Certain post

processing techniques can also be employed to manipulate images to better visualize the pathology of concern.

MRI is known in the medical community to be a relatively safe form of medical imaging because it does not utilize ionizing radiation. Magnetic resonance imaging instead utilizes extremely high strength magnetic fields and time varying electromagnetic waves oscillating in the radio frequency range of the electromagnetic spectrum. It is however necessary that a human subject is thoroughly screened regarding metallic devices before the MRI scan to prevent harm being placed in a very strong magnetic field. Safety is of the utmost concern when designing, and carrying out a research study involving magnetic resonance imaging.

All this information is useful when beginning to understand the process by which accurate fat fraction percentage maps are created. The varying pulse sequences and MRI scanner parameters which are utilized to create these fat percentage maps are of intrinsic importance to the research being undertaken. It is invaluable to have a working knowledge of MR basics when attempting to analyze any human anatomy via non-invasive methods at the highest level. The following sections will detail how MR analysis is manipulated to allow for accurate fat quantification in a much more accurate way when compared to biopsy. As well as in manners which will take into account the entirety of the liver whereas biopsy only takes into account a miniscule sample size.

REVIEW OF LITERATURE: “CURRENT EFFORTS AND TRENDS IN THE TREATMENT OF NASH” (RIZIU ET AL)

It is useful that we review this article because through examination of this text we are able to more clearly understand future directions regarding efforts in pharmacological treatment for this disorder as well as limitations holding back the pace of research. Currently there is not an approved pharmacological treatment for Non-alcoholic steatohepatitis which is an intrinsic facet of nonalcoholic fatty liver disease. In this literature Ritziu et al explore reasons that have perhaps hindered the advancement of NASH therapies in the pharmacological realm. Also examined, is the current state of research in non-pharmacological and pharmacological management of NASH treatments.

Ritziu et al note that in the study of nonalcoholic fatty liver disease, little attention in the past has been paid to NASH, because steatosis was most commonly seen as a benign lesion, or a side effect of obesity or diabetes. It is worth noting that most diabetologists will not see their diabetic subjects when they present with cirrhosis. It is also a commonly held misconception among those researching this disorder that NASH is a complication of diabetes, and that antidiabetic drugs will treat NASH making a specialized treatment of NASH not needed. This is shown to be untrue though because this reasoning doesn't take into account the importance of the fact that those who suffer from NASH are not within the cohort of those with uncontrolled diabetes. Also it is noted by Ritziu et al that the top most commonly utilized drugs in type two diabetes are not necessarily effective for NASH. Also

there is a notion within the medical community that because those who suffer from NAFLD are overweight then diets and exercise, as opposed to pharmacological intervention, are more appropriate and cost effective treatment options. This however does not prove effective due to the limited ability of hepatological centers to control patient lifestyle choices. Therefore it is indeed prudent and necessary to initiate clinical trials in this research sector. Others have recommended that clinical trials regarding NASH may not be practical because of cost constraint involved with biopsy for histological documentation. But there have been developments in this sector involving several phase 2b trials which have begun and were able to recruit fully. This shows us that there is significant enough medical need to overcome the limitation presented by liver biopsy.

Ritzui et al next delve into an explanation of the pathology of NASH in order for us to better understand the mechanism behind this disorder and how it may be combatted. The disease is usually initiated through diet or lifestyle induced caloric overload, or in some cases drugs. After the disease is initiated there is a multitude of cellular processes which come in to play. These are progressive inflammation, cell death, and fibrogenesis. In response to this tissue repair pathways are also activated, and will have the effect of restoration of tissue and metabolic homeostasis. This balancing act between progression of disease and the healing of tissue will influence whether or not cirrhosis will occur. The histological definition of NASH includes the lobular inflammation, the occurrence of steatosis, and hepatocyte ballooning with a centrilobular pattern. The following challenges which

face the furthering of research in this field are beginning to see progress. The fact that there are several pathogenic mechanisms at play is responded to by the recognition of medical need. The imperfect animal model research has seen progress in the fact that NASH as an indication for therapy is accepted. An operational pathological definition of NASH has met the challenge of non-invasive proof needed for principle trials. Agreed upon and achievable surrogate endpoints are surrogates for hard outcomes.

Next Ritziu et al look into the management of NAFLD, and lessons that have been learned to date. We know that through animal models high fat diet alone can induce steatosis, whereas high fat diet plus high sucrose diets induce inflammation, steatohepatitis, fibrosis, and oxidative stress. The question is presented: can extensive fibrotic NASH when only alterations are made in a patients diet and exercise. It is also worth noting that bariatric surgery may also correct the pro-inflammatory state which is associated with obesity.

REVIEW OF LITERATURE: “ESTIMATION OF HEPATIC PROTON-DENSITY FAT FRACTION BY USING MR IMAGING AT 3.0 T” (YOKOO ET AL)

This paper focuses on analyzing liver fat fraction by comparing several magnetic resonance techniques against MR spectroscopy (which is utilized here as the reference technique), in a population of one hundred and sixty-three volunteers. The breakdown of the subjects is as follows: thirty-nine of the subjects had diagnosed fatty liver disease, one hundred and ten had risk factors for developing steatosis, and fourteen did not have any known risk factors. The risk factors are documented by Yokoo et al as being a body mass index of twenty five kilograms over meters squared or greater, hepatitis C diagnosis, first degree relative who has hepatic steatosis, and elevated levels of alanine aminotransferase serum. It is worth noting that alcohol and medication usage history was not analyzed as a confounding factor. Yokoo et al define hepatic steatosis as “excessive triglyceride (fat) accumulation in hepatocytes”, and note that it is one of the most common pathologies present in alcohol and nonalcoholic fatty liver disease. One of the focuses of this study is the analysis of noninvasive magnetic resonance techniques, because unlike liver biopsy, they are much more objective and the whole liver is able to be analyzed as opposed to a particular section.

Magnetic resonance spectroscopy is a particularly valuable tool for calculating liver fat fraction. This is due to the distinct frequencies at which multiple proton-containing chemical moieties resonance within the triglyceride molecule.

However, this way of measuring liver fat is not feasible on a multi-voxel approach, and is most successful on a single voxel approach. Unfortunately, a single voxel approach is not feasible for longitudinal studies due to variability and sampling errors. Magnetic resonance imaging of the entire liver is much more accessible in a clinical setting; however, several factors need to be taken into account in order to produce accurate images. For example, a non-T1-weighted sequence can be utilized to combat the effect of unequal water and fat T1 times. Also, T2* related signal loss can be overcome through the use of several echo times. T2* is the time constant for loss of phase coherence among spins oriented at an angle to the static magnetic field due to a combination of magnetic field inhomogeneities and the spin-spin relaxation. Results in a rapid loss of transverse magnetization and the MRI signal. In this study 3.0 Tesla MR techniques are tested against MR spectroscopy which is utilized as the technique of reference because it provides a more direct measurement of fat content. In particular, the advantages and drawbacks of T2* correction techniques and multiple frequency models were assessed.

For the spectroscopy portion of this study a single voxel measuring 20x20x20 mm was taken using MR imaging with a very long TR of 3500 ms to minimize T1 weighting. A single pre-acquisition pulse was applied to balance T1 saturation on following excitations. Five stimulated echo acquisition mode spectra were taken at echo times of ten, fifteen, twenty, twenty-five, and thirty ms.

For the magnetic resonance imaging portion of this study a two-dimensional gradient echo pulse sequence was utilized. In order to minimize T1 weighting, a low

flip angle of ten degrees was used by Yokoo et al. Six fractional echo magnitude images were taken at echo times of 1.15, 2.30, 3.45, 4.60, 5.75, and 6.90 m. As part of post processing a circular region of interest was placed on the voxel location from the spectroscopic portion of the study. In order to calculate the proton-density fat fraction the fat proton density, which was the sum of all the fat peaks, was divided by the sum of the fat and water proton densities. The fat fraction of the ROI in the MR portion of the study was calculated by using the two, three, or six echo times. The three and six echo methods took into account all three confounding factors of T1, T2*, and multiple frequencies. The result of these two imaging techniques being administered was that each subject in the study had one imaging and one spectroscopic fat measurement. If time allowed, some subjects underwent extra acquisitions, which were then utilized to assess overall estimation accuracy and classification.

The main result of this study is that magnetic resonance imaging is able to come close to spectroscopic fat fraction if certain effects are taken into account and corrected. T1 weighting must be minimized, T2* must be corrected for, and multiple signal frequencies must be modelled to elicit the most accurate results possible. It is important to note that Yokoo et al utilized spectroscopy, instead of histology, as the technique of reference due to the following reasons. For one, fat fraction which is calculated from spectroscopy determined by densities of protons is a direct correlation to the content of triglyceride in fat molecules. Also, both MR and spectroscopy are utilized to analyze fat levels volumetrically by looking at the

relative amount of fat protons. It is also worth noting that up to three different segments of the liver were analyzed as part of this study. This portion of the study resulted in close relation between imaging and spectroscopy in the various sampled segments which tells us that there is a strong degree of accuracy across the entirety of the liver.

This study concludes by stating that magnetic resonance imaging at 3.0 Tesla is a viable and accurate measure of hepatic fat content. This finding will allow the research community to utilize MR imaging as an alternative to spectroscopy when it comes to hepatic fat quantification. The statistical analysis of MR versus spectroscopy of hepatic proton density fat fraction led to Yokoo et al formulating this conclusion.

**REVIEW OF LITERATURE: “QUANTITATIVE ASSESSMENT OF LIVER FAT
WITH MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY”
(REEDER ET AL)**

This paper begins by defining hepatic steatosis as the “accumulation of lipid within hepatocytes”, and notes that it is “the histological hallmark of non-alcoholic fatty liver disease”. Triglycerides are the most common form that lipid vacuoles will take within hepatocytes. Hepatic steatosis is extremely common, and in the United States nonalcoholic fatty liver disease has an epidemiological occurrence of affecting twenty to eighty million Americans. This makes NAFLD the most commonly occurring chronic liver disease in the US. Reeder et al note that a small percentage of those who present with NAFLD will eventually progress to cirrhosis and perhaps over time hepatocellular carcinoma. Twenty to fifty percent of individuals with steatosis will eventually develop diabetes. This is due to hepatic steatosis causing interference in insulin signaling pathways, resulting in insulin resistance in the liver.

The first section of this paper by Reeder et al looks in to the diagnosis of hepatic steatosis. Quantitative measurement is necessary and important for understanding the severity of this disorder in both a clinical and research setting. Longitudinal studies rely on the ability of various imaging techniques to make an accurate measurement of hepatic fat content. Qualitative measures such as liver biopsy rely on visual analysis of hepatocytes which contain vacuoles of fat. This results in significant variation in grading between individuals. Another drawback of this measurement technique is that it is done in two dimensions. Liver fat

triglyceride levels can be analyzed with biochemical assay, but the tissue which is used for this is destroyed and cannot be analyzed histologically. The non-uniform nature of liver fat accumulation in the liver also presents an inherent issue for biopsy which takes a very small sample and only represents that samples hepatic fat content.

A significant portion of this article next focuses on why a statistically significant cutoff (percentage of liver fat) is necessary for hepatic clinical and research based studies in order to provide a meaningful cut off point for hepatic fat content assessment in both clinical trials and research studies. Reeder et al that there was a seminal study done by Szczepaniak et al in Dallas which stated over 5.56 percent liver fat was abnormal in patients that presented without an identifiable risk for hepatic steatosis (Szczepaniak et al). If the cut off value is low then this would be most appropriate for screening for clinical studies, because there would a greater sensitivity to slightly above average levels of liver fat. If the cut off value defined by study parameters as being higher, this would be generally better for actual implementation of therapies and procedures, “exercise vs drugs vs bariatric surgery vs observation only”. It is important that standard deviation is significantly lower than the cut off percentage number. The number of patients required to show efficacy in clinical trials will be reduced by further proof a statistically significant imaging biomarker for NAFLD.

The most common modality utilized to detect hepatic steatosis is ultrasound, due to its relative low cost and convenience. There are however several drawbacks

of using ultrasound for this purpose. For one, “fibrosis, edema, and extrahepatic adipose tissue”, can create beam attenuation and echotexture. This is not appropriate for quantification in a clinical or research setting. Another modality with significant drawbacks when it comes to efficiency and accuracy for hepatic fat quantification is CT. CT utilizes ionizing radiation via an x-ray beam to measure variations in beam attenuation. The “presence of iron, copper, glycogen, fibrosis, and edema”, all negatively affect the accuracy of measuring liver fat.

Noninvasive MR measurement of hepatic steatosis is most commonly in the form of magnetic resonance imaging and spectroscopy. These both inherently rely on the difference between water and fat proton resonance frequencies to quantitatively measure liver fat. The fat fraction in magnetic resonance imaging refers to the signal coming from the liver which we can attribute to fat, while in magnetic resonance spectroscopy proton density fat fraction refers to the “fraction of mobile protons in liver attributable to fat”. The first and most crucial step in calculating MR signal or proton density fat fraction is decomposing the signal into a fat and water component. The fat fraction signal is calculated by dividing the signal from fat by the combination of the signal from water plus fat. Due to the ratio nature of this calculation, the influence of RF coil sensitivity is corrected for. This calculation in spectroscopy occurs at the level of a single voxel, while in MR imaging, it occurs pixel-by-pixel to create a parametric map of fat fraction. Reeder et al state that because of the confounding factors including “T1 bias, T2 relaxation, T2* decay,

spectral complexity of the fat spectrum, J-coupling, noise bias, and eddy currents”, fat fraction is not necessarily a reliable measure of fat content in the liver.

Magnetic resonance spectroscopy is described as being an accurate way of parsing the signal from the liver in to its fat and water components in order to eventually calculate the fat fraction. This is done using single voxel imaging, and taking care to avoid the edge of liver and also vessels and bile ducts. The two sequences predominantly used as stimulated echo acquisition mode, and point resolved spectroscopy. It is crucial that neither fat or water saturation are employed when using either of these sequences. The water spectrum is easily identified due to the hydrogen protons of the water molecule having only the same frequency, whereas the fat molecule has nine different proton moieties, each of which have a unique frequency. The fat fraction is calculated as the area of the fat peak, divided by the areas of water and fat peaks added together.

Fat suppression techniques which rely on selective water excitation, allow us to separate the liver magnetic resonance signal into water and fat components by looking at the difference between images which are not fat suppressed versus those which have been fat suppressed. Image signal which has been fat suppressed is assumed to represent the water signal, whereas non-fat suppressed signal is assumed to represent water plus fat signal. This technique has not been reliably used as a way to measure proton density fat fraction due to the difficulty in achieving complete fat saturation in a homogeneous nature across the liver.

Chemical shift imaging techniques work by separating the magnetic resonance

signal in to fat and water components “by acquiring images at two or more echo times (TEs) after signal excitation”. Reeder et al discuss two approaches: one which utilizes magnitude data only (not including phase information), as well as a separate approach which uses complex data (using both magnitude and phase information). The magnitude-based chemical shift approach works by acquiring two gradient echoes, one when the water and fat peak are out of phase, and one at a TE when the two peaks are in phase. This is commonly known as dual echo imaging. Signals will be in phase every 4.6 m, because at 1.5 T the chemical shift between water and the main fat peak is 3.4 ppm which corresponds to a frequency difference of -217 Hz. The signals are out of phase at 2.3, 6.9, 11.5 m. At 3T these echo times are halved. In phase 2.3, 4.6, 6.9 m, out of phase 1.15, 3.45, 5.57 m. The signal intensity from the Out of Phase images represent the difference between the water and fat signal, while in phase represents the sum of fat and water signals. Therefore, the fat fraction percentage is equal to the signal from In phase minus out of phase, divided by two times the signal from in phase. The complex method, which utilizes phase as well as magnitude, works through the acquisition of three or more echo times. As opposed to the magnitude based approach, this complex method allows for the complete distinction between fat and water signals in order to achieve a range that is on the scale of 0 to 100% fat fraction percentage.

Next Reeder et al discuss several factors that could affect the fat fraction signal. The first discusses involves the relative enhancement of the signal from fat, compared with the signal from water, when the images are T1 weighted. In

magnetic resonance spectroscopy, a long repetition time is often utilized to combat this, where as in magnetic resonance imaging a low flip angle may be used. Another bias involves the signal from T2 being relatively enhanced in the fat signal, compared with the water signal. This is because the signal from fat is the longer of T2 times, while the signal from water is shorter. One strategy to counteract this is to use the lowest echo time possible. Another way to combat this is to use population based water and fat values in order to correct for T2 decay. T2* decay results in greater signal loss at the TE increases in chemical shift based MRI fat quantification methods (which acquire imaging at increasing echo times following RF excitation). T2* correction is done by combining T2* in the signal model which is used to calculate the fat fraction. You can also measure T2* separately and correct for the effects of T2*. By measuring T2* simultaneously with water and fat signal the confounding effects on signal decay can be avoided. Noise bias can be another confounding factor which may occur during the combination of separate fat and water images. These are more prominent in chemical shift based fat vs. water separation methods which deal with low fat-fractions near zero.

In summary Reeder et al acknowledge that biopsy is the current gold standard for assessment of hepatic steatosis, however is has multiple negative factors that make it not ideal for assessing the severity of steatosis, nor does it allow for accurate longitudinal study. Due to the confounding factors discussed above, MR based signal fat fraction is not necessarily an appropriate biomarker because it depends so heavily on platform and scan parameters.

DISCUSSION

This thesis paper draws on research regarding nonalcoholic fatty liver disorder from a variety of standpoints. It is important to have a complete view of the extent of nonalcoholic fatty liver disease in order to understand the importance of developing non-invasive methods of accurately measuring levels of fat in the liver. By verifying techniques for fat fraction map creation, the science community is able to agree on standards for defining and diagnosing fatty liver disorder in populations. This is of particular importance to clinical trials, which rely upon longitudinal studies to quantify the efficacy of drugs in treating this disorder by diminishing the levels of fat accumulation in the liver. The three papers discussed above each elucidate important aspects of the research being done in regards to this indication. Ritziu et al looks specifically at nonalcoholic steatohepatitis and the acute need for a biomarker which will monitor disease progression or recovery resulting from treatment methodologies. They do note that of all current pharmacological treatments in trial for the treatment of NASH, there are none which are currently set to make a marked improvement. Therefore, it is prudent that the scientific community dedicated to this disease continue to research and identify new targets for therapy. It will also be helpful in doing so to combine synergistic pathways in the treatment of NASH. Finally, Ritziu et al note that it will be prudent to research therapies that involve a more individualized approach based upon response to treatment and the severity of the disease. In doing so perhaps anti fibrotic and anti-NASH treatment agents will begin to emerge in the clinical research community of nonalcoholic fatty liver disease.

Yokoo et al show through a clinical study that hepatic proton density fat fraction measurements taken with accurate imaging at 3.0 tesla magnetic resonance are accurate when compared to magnetic resonance spectroscopy. This is an extremely important finding because magnetic resonance imaging allows the researcher to holistically view the entire liver whereas spectroscopy is limited to one small voxel. Discrepancies in how liver fat is distributed across the liver make it necessary for the entire liver to be analyzed when deciding upon a biomarker to be used for liver fat quantification levels. Magnetic resonance imaging is much more readily available in the hospital setting, as well the world of clinical trials, making it a much better future alternative to quantifying liver fat percentages in the future. Finally, the Reeder et al study which has been analyzed in this paper is a comprehensive look into the variety of techniques which are currently utilized for quantifying liver fat percentages, and creating liver fat fraction maps. Through analyzing the pros and cons of each of these techniques, Reeder et al are able to accurately elucidate the methods by which scientists and researchers can assess liver fat percentages via fat fraction maps. Reviewed together these three papers, along with the epidemiological data surrounding the prevalence of fatty liver disease, we are able to assess the severity of this disease and the dire need for an accurate method of non-invasively measuring the percentage of a patient's liver fat. This will allow for the accurate and cost effective diagnosis of this disease in the hospital setting, as well as the quantification of how the severity of the disease reacts to pharmacological treatment in clinical trials. Through increased research in this area, the magnetic resonance research community may eventually be able to define biomarkers by which disease severity can be

assessed. This will help the overall population of those who suffer from this disease, and ideally help to curb this disease in the United States and around the world.

LIST OF JOURNAL ABBREVIATIONS

Am J Physiol	American Journal of Physiology
Gastroenterol	Gastroenterology Journal
J Hepatol	Journal of Hepatology
Mayo Clinic Proc	Mayo Clinic Proceedings
N Engl J Med	New England Journal of Medicine
Pediatric Ann	Pediatric Annals
World J Gastroenterol	World Journal of Gastroenterology

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