



Magnetic resonance imaging fatty liver changes following surgical, lifestyle or drug treatments in obese, non-alcoholic fatty liver disease or non-alcoholic steatohepatitis subjects

With an increasing insulin resistance and obesity epidemic, various intervention trials aiming to reduce liver fat levels are using non-invasive MRI methods as alternatives to repeated biopsies to measure the changes in liver fat levels in controlled clinical trials. MRI and MRS exploit differences in the resonance frequencies of signals from protons in fat and water to quantify liver and muscle fractional fat content or fat depot volumes in a highly reproducible manner. Various clinical trials have reported the baseline liver fat values and ranges along with the changes in fat levels following different surgical, nutritional, lifestyle or pharmaceutical interventions.

KEYWORDS: insulin ■ non-alcoholic fatty liver disease ■ diet ■ MRI ■ steatosis

Introduction

The liver is the largest and heaviest internal organ in the body. The liver filters blood coming from the gut before distribution to the rest of the body while metabolizing drugs and detoxifying chemicals for elimination. The liver serves as a storage organ as well, managing fat, iron and glycogen content within hepatocytes [1]. Fat accumulates in the liver for many reasons, the most common cause of fat accumulation is metabolic dysregulation often associated with obesity and insulin resistance [2]. Other factors can also cause fat to accumulate in the liver, including alcohol [3], steatogenic drugs [4], some viral infections [5] and rare genetic disorders [6-8]. This review will focus on obesity- and insulin-resistance associated-metabolic dysregulation.

Liver fat has recently been a target of various types of interventions designed to reduce the amount of lipid droplets sequestered within the hepatocyte. The clinical goal is simple: reduce metabolic stress and liver fat levels before the path of inflammation and fibrosis becomes irreversible.

The liver is quite amenable to clinical imaging methods, which may serve as ideal companions or replacements to liver biopsy which only samples a small portion. Ultrasonography (US), magnetic resonance imaging (MRI), and spectroscopy (MRS) [9] have recently been reviewed by Lapadat et al. [10] with dual-energy X-ray absorptiometry (DXA) used by Monteiro et al. [11]. In a B-mode ultrasound exam, the

normal liver, spleen, and kidney have similar echogenic textures, but when intracellular liver fat accumulates, hepatic echogenicity increases relative to these comparator organs; additionally, the ultrasound beam becomes attenuated causing blurring of intrahepatic vessels and obscuration of deep structures [10,12]. Differences in tissue densities, or more appropriately, the ability to attenuate X-rays, are how computed tomography (CT) generates its contrast. CT attenuation is measured by Hounsfield Units with normal liver higher than spleen, and fatty liver values lower than normal; so as liver fat increases, there is a commensurate decrease in quantitative Hounsfield Units [10]. MRI and MRS exploit differences in the resonance frequencies of signals from protons in fat and water to quantify fractional fat content in a highly reproducible manner [13]. MRS methods can detect liver fat with exquisite sensitivity but usually from only a small liver volume, often in the right lobe devoid of major vessels. [14-28]. Skeletal muscle spectra are often derived from lower limb muscles, such as the soleus or anterior tibialis [15,19,20,24] or thigh [29]. MRI methods using the 2-pt. Dixon [30,31] and 3-pt. Dixon [32,33] methods are being replaced by the superior MRI proton-density fat-fraction (MRI-PDFF) acquisition protocol [29,34-37]. In measurements of subcutaneous and visceral fat depots, regions of interest are segmented from registered MRIs, generating areas or volumes based on voxel parameters [14,28,29,31,33,38].

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Applying an imaging method in a clinical trial designed to measure a liver fat effect requires several considerations. What level of liver fat is necessary for inclusion? How much of a change from baseline is clinically relevant and predicts or reflects a beneficial clinical outcome? How many modalities or measures are needed to demonstrate that the clinical effect is real? What if the measurements do not affect the disease outcome?

These questions could easily be applied to any drug or intervention in a clinical trial, but this review will focus on published reports pertaining to treatment in fatty liver subjects.

We surveyed the literature, focusing on PubMed clinical trial reports from 2000 to April 2017 that reported treatment results in subjects with elevated liver fat as assessed predominantly by MRI or MRS methods. Pre-clinical trials were omitted, as

Table 1. Summary of surgical clinical trial populations, design and treatment findings.

First Author (et al.) ¹	Year	Subject Population	Enrolled subjects ²	% Liver fat at inclusion or baseline ³	Range ⁴ (est.)	Intervention Design	Treatment	Duration	Trial Findings ⁵
Johansson	2008	Morbidly obese	7	14.4% (MRS)	(6.3-22.6)	Prospective, self-controlled	Roux-en-Y gastric bypass surgery	12 month	Reduction in body weight (-34.4%); visceral fat depot was reduced at 1 month (-28.4%), 6 months (-52.8%), and 1 year (-69.7%); subcutaneous fat depot was reduced at 1 month (-10.1%), 6 months (-36.5%), and 1 year (-55.3%); liver fat levels were reduced 6 months (-57.4%), and 1 year -87.2%); liver volume were reduced 6 months (-21.8%), and 1 year (-29.9%); extramyocellular lipid levels were reduced 6 months (-30.6%), and 1 year (-39.0%) while intramyocellular lipid levels were not different
Heath	2009	Morbidly obese	18	24% (MRS)	(19-29)	Prospective, self-controlled	Laparoscopic adjustable gastric banding surgery	12 month	Reduction in body weight 3 months (-9.3%) and 1 year (-19.0%), visceral fat depot was reduced at 3 months (-21.7%) and 1 year (-37.1%), subcutaneous fat depot was reduced at 3 months (-14.4%) and 1 year (-29.1%), liver lipid levels were reduced at 3 months (-36.4%) and 1 year (-45.5%)
van Werven	2011	Morbidly obese	38	5.8% (median MRS)	(0.4-30.5)	Prospective, self-controlled	Roux-en-Y gastric bypass surgery	3 month	Hepatic fat content decreased significantly from 5.8% (median, range -0.1–21.4%) to 3.1% (median, range -7.5–27.2%), a magnitude of -46.6%
Calvo	2015	Obesity-induced NAFLD	19	30% (MRI)	(5-55)	Observational, prospective, cross-sectional study	Laparoscopic sleeve gastrectomy	12 month	BMI and liver fat were significantly reduced (-28.9% and -80.0%, respectively)

Footnotes

¹References are arranged by publication date.

²Screened, enrolled subjects, any modification to the enrolled pool used in analysis are derived from the methods section of each paper.

³Baseline or liver fat value required for inclusion is listed, along with the method used to make this assessment.

⁴Ranges are from the methods section or derived to approximate a 95% confidence interval (CI) using the SD or standard error of the mean (SEM).

⁵Trial Findings include the absolute change in measurements along with its magnitude.

were human trials which did not report baseline and follow-up treatment hepatic fat fraction values in tables or figures. In general, subjects were often adults (ethnic composition of subject population often was not reported unless specifically tested), overweight or (morbidly) obese, non-cirrhotic, often presenting with a form of non-alcoholic steatosis, with varying levels of insulin resistance. The clinical trial designs were predominantly randomized self-controlled investigations from single sites. For the purposes of the review, we've organized these fatty liver investigations into three main areas: surgical interventions designed to

control obesity (TABLE 1), lifestyle changes such as diet and exercise designed to reduce weight or increase insulin sensitivity (TABLE 2), and pharmaceutical interventions targeting specific molecular pathways in subjects with fatty liver for various reasons (TABLE 3). The latter two groups included obese subjects, many with type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH). What many of these trials share is that a subject population was selected and a serial imaging strategy was applied as part of the experimental design. The fatty liver levels in the intended study populations at

Table 2. Summary of lifestyle clinical trial populations, design and treatment findings.

First Author (et al.) ¹	Year	Subject Population	Enrolled subjects ²	% Liver fat at inclusion or baseline ³	Range ⁴ (est.)	Intervention Design	Treatment	Duration	Trial Findings ⁵
Thomas	2006	NAFLD	10	13.3% (MRS)	(5.8-30.9)	Prospective, self-controlled	Diet and exercise	6 month	Modest weight loss, reduced liver fat (7 of 10, -39.8%), subcutaneous adipose, and intra-abdominal adipose; variable effects on intramuscular lipid
Thamer	2007	Healthy, non-diabetics at risk for type 2 diabetes	112	6.5% (MRS)	(5.8-7.2)	Prospective, self-controlled	Diet and exercise	~9 month	Significant, but small loss of body weight (-3%), major reductions in visceral fat mass (-12%) and 7th segment liver fat (-33%)
Johnson	2009	Obese, sedentary, non-alcoholics	19	8.7% (MRS)	(0-41.6)	Prospective, computer randomized to aerobic exercise or stretching (placebo)	Exercise vs. stretching	4 week	No differences in visceral or subcutaneous fat depots, exercise reduced liver fat (-20.6%) compared to stretching (+2.8%)
van der Heijden	2010	Hispanic adolescents	29	5.6% (MRS)	(1-15)	Prospective, controlled (obese vs. lean body fat)	Aerobic exercise program	12 week	Visceral and liver fat within groups was reduced in obese but not lean subjects -9.3% and -37.1%, respectively, with differences between groups; subcutaneous fat and intramyocellular fat were not different within or between groups
Pozzato	2010	Obese children (6-14 YO)	26	9% (2-pt. Dixon MRI)	(0-36)	Prospective, self-controlled	Nutrition-behavior intervention	1 year	Reduced BMI z-score (-12.7%), reduced liver fat (-63.5%)

Bjermo	2012	Abdominally obese subjects	67 → 61	7.2% (MRI)	(4.1-15.7)	Randomized, controlled	Fatty acid diet modification	10 week	Body weight change, visceral, subcutaneous, or total fat mass depots, within or between groups, at baseline, or after diet modification, were not different; liver fat differences between diet groups were +9.3% (MRI) and +9.4% (MRS)
Bacchi	2013	Type 2 diabetics with NAFLD	40	28.5% (2-pt. Dixon MRI)	(5.9-51)	Randomized, controlled	Aerobic or resistance training groups	2 month diet lead-in, 4 month physical activity treatment	Reductions in all adipose depots and liver fat (-37.7% aerobic, -38.2% resistance) content from baseline, but not between exercise group
Haufe	2013	Overweight and obese subjects without signs and symptoms of concomitant disease	50	7.6% (MRS)	(0-25.6)	post-study follow-up	6 month hypocaloric diet, long-term follow-up assessment	Long-term follow-up	Significant decrease with rebounding increase in visceral (-20.3% No NAFLD, -20.3% with NAFLD at 6 months, -15.1% No NAFLD, -6.6% with NAFLD at follow-up) and subcutaneous (-16.8% No NAFLD, -15.1% with NAFLD at 6 months, -7.4% No NAFLD, -7.5% with NAFLD at follow-up) abdominal fat masses, liver fat decreased during the dietary intervention and remained reduced at follow-up (-35.5% No NAFLD, -44.3% with NAFLD at 6 months, -16.1% No NAFLD, -49.1% with NAFLD at follow-up)
Argo	2015	Non-cirrhotic NASH	33	Steatosis with inflammation, hepatocellular ballooning, and/or fibrosis (biopsy); 14.8% (2-pt. Dixon MRI)	(0-32)	Randomized, double-blind, placebo-controlled	n-3 polyunsaturated fatty acid fish oil vs. counselling	1 year	n-3 fish oil treatment reduced liver fat and but not abdominal fat (-54.3% and -25.8%, respectively); counselling alone did not reduced liver fat or abdominal fat (+6.2% and -12.3%, respectively), NASH Activity Score was not different

Pacifico	2015	Children with NAFLD	51	14.8% (3-pt. Dixon MRI)	(8.0-23.0)	Randomized, double-blind, parallel-group, placebo-controlled	Docosahexaenoic acid (algae oil) vs. linoleic acid (placebo)	6 month	Docosahexaenoic acid treatment reduced liver (-53.6%), visceral (-7.8%) and epicardial (-14.9%) adipose, but not subcutaneous adipose tissue depots
Adams	2015	NAFLD	74 → 61	18.3% (3-pt. Dixon MRI)	(0-41.4)	Prospective, randomized, controlled	venesection with lifestyle advice vs. lifestyle advice alone	6 month	No difference in liver fat or iron levels
Cuthbertson	2016	NAFLD	69	17.7% (MRS)	(11.7-39.0)	Randomized, controlled	Supervised exercise vs. counselling	16 week	Median liver fat (MRS) was significantly reduced in the exercise group (-47.9%) but not the counselling group (-8.8%), with a significant absolute difference between group means (-4.7%); subcutaneous and visceral adipose tissue depot mean changes were significant in the exercise group (-10.4% and -12.2%, respectively), significant difference in group means for subcutaneous adipose (-10.4%)
Agebratt	2016	Healthy non-obese	30	2.1% (MRI-PDF)	(1.4-2.9)	randomized	Extra fruit vs. extra nuts	2 month	Nut group gained body weight, no changes in liver, subcutaneous, or visceral adipose depots

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baseline along with potential changes over time will be useful to investigators designing clinical trials with fatty liver endpoints. The purpose of this review is to collate the MRI/MRS fatty liver results from various subjects and interventions for consideration in future clinical trial designs.

■ Surgical intervention

To be classified as morbidly obese, an individual must be at least 100 pounds over his or her ideal weight, with a body mass index (BMI) above 40, or a BMI above 35 with an accompanying morbidity, such as hypertension, dyslipidemia or type 2 diabetes. In such subjects, the body fat mass is typically expanded with excess fat deposition in multiple adipose depots

and organs, including the liver and skeletal muscle. Roux-en-Y gastric bypass is a common and successful bariatric surgery procedure, and Johansson et al. [20] documented the metabolic and physical improvements in seven women having the procedure at 1, 6 and 12 months post-surgery. This investigation used magnetic resonance imaging (MRI) to assess visceral and subcutaneous adipose depots and liver volume and magnetic resonance spectroscopy (MRS) to assess liver fat levels and intra- and extra-myocellular lipid levels. Even with a small number of subjects, gastric bypass produced a mean 12 month reduction in body weight of -34.4% with an accompanying -34.3% BMI reduction. The visceral fat depot was reduced

Table 3. Summary of pharmaceutical intervention clinical trial populations, design, and treatment findings.

First Author (et al.) ¹	Year	Subject Population	Enrolled subjects ²	% Liver fat at inclusion or baseline ³	Range ⁴ (est.)	Intervention Design	Treatment	Duration	Trial Findings ⁵
Arioglu	2000	Various syndromes associated with lipotrophy or lipodystrophy	20	23.6% (total fat, DXA)	(7-43.6)	Open-label prospective	Troglitazone	6 month	Increased total fat (+2.4%, DXA), liver volume decreased (-12.5%, MRI)
Carey	2002	Type 2 diabetes	33	25.1% (relative to water, MRS)	(4-42.8)	Double blind placebo controlled	Rosiglitazone	16 week	Increase in body fat was not uniform, increase in abdominal fat occurred in the subcutaneous but not visceral adipose, rosiglitazone exposure decreased the mean liver fat from +22.0% to 15.3%
Promrat	2004	NASH	18	47.5% (MRI)	(19.6-75.4)	Prospective, self-controlled	Pioglitazone	3 month pre-treatment period, 48 week treatment	Liver biopsies improved, NASH activity score decreased, patients gained weight, increase total fat mass but no change in lean body mass (DXA), decreased liver fat (-52.0%) and liver volume (-13.8%, MRI)
Javor	2005	Generalized lipodystrophy	10	31% (MRI)	(24-38)	Open label, self-controlled	Recombinant methionyl human leptin	4-8 month	Biopsy improvements in steatosis and ballooning injury, fibrosis was unchanged, mean NASH activity was a reduced; MRI assessments of liver fat (-64.5%) and liver volume (-25.5%) indicate reductions after treatment
Lutchman	2006	Non-alcoholic steatohepatitis	18	>47.5% (biopsy)	(19.6-75.4, MRI)	Prospective, self-controlled	Pioglitazone	3 month pre-treatment period, 48 week treatment	No correlation between initial adipokine levels and liver biopsy NASH scores, adiponectin or leptin levels did not correlate with liver fat (histology or MRI), liver fibrosis did not correlate with proinflammatory cytokines, leptin or adiponectin serum levels, potential correlation between serum leptin level and the NASH activity index

Juurinen	2007	Type 2 diabetes	14	17% (MRS)	(14-20)	Prospective, self-controlled	Insulin	7 month	Body weight increased, liver fat decreased (-17.6%, MRS), fat distribution and fat mass remained unchanged (MRI)
Zib	2007	Type 2 diabetes	32	9.5% (fat/water, MRS)	(0.0-20.0)	Prospective, randomized, open-label study	Pioglitazone added to insulin	2 week lead-in period, randomization, 6 month treatment	Subjects gained weight, subcutaneous fat mass increased, visceral fat mass remained unchanged and average liver fat content significantly Decreased after pioglitazone plus insulin treatment (-52.1%) compared to the insulin – only comparator group (-24.1%)
Ravikumar	2008	Type 2 diabetics on sulfonylurea therapy	10	140.1 μ mol/g (NMR)	(112.0-168.2)	Prospective, self-controlled	Pioglitazone	4 week run-in period, 16 week treatment	Increases in body weight and subcutaneous fat; no change in total visceral fat content but a decrease in visceral fat-to-subcutaneous fat ratio, decrease in liver fat content (-52.2%)
Eriksson	2008	Non-diabetic subjects with hypertension and abdominal obesity	35 \rightarrow 22	4.8% (MRS median)	(2.3–9.9)	Double-blind, placebo run-in (n=35), 3-way cross-over (n=22)	Hydrochlorothiazide, Candesartan	36 week	Reduced blood pressure, no differences in abdominal obesity or body fat percentage, liver fat content and volume were greater with hydrochlorothiazide treatment, no differences in intra- or extramyocellular lipids
Le	2012	Biopsy-proven NASH	50	16.1% (MRI-PDFF)	(7.9–25.6)	Randomized, double-blind, allocation-concealed, placebo-controlled	Colesevelam	24 week	Colesevelam treatment increased liver fat (+19.7% and +15.3%, MRI-PDFF and MRS, respectively) compared to placebo liver fat measures (-15.1% and -14.4%, MRI-PDFF and MRS, respectively)
Chachay	2014	Overweight or obese men diagnosed with NAFLD	20	25.5% (MRS)	(0-60.8)	Randomized, double-blind, placebo-controlled	Resveratrol	8 week	Treatment did not change body weight, visceral (-2.3% resveratrol vs. -6.5% placebo) or subcutaneous (+1.4% resveratrol vs. +2.1% placebo) adipose depots, or liver fat (+8.0% resveratrol vs. -3.8% placebo)

Loomba	2015	Biopsy-proven NASH	50 → 45	≥ 5% (MRI-PDFF)	(5-35.6)	Randomized, double-blind, allocation-concealed, investigator-initiated, placebo-controlled	Ezetimibe	24 week	MRI-PDFF liver fat was reduced in the ezetimibe group from baseline (-22.7%) but not from the placebo group (-11.4%) and confirmed by MRS (-22.3% and -9.9%, respectively; r2=0.95-0.99); no between arm or treatment difference in liver fibrosis (MRE)
Kato	2015	Overweight Japanese subjects with type 2 diabetes	20	Difference between the echo intensities of the liver and kidney (GSUS); 36.5% (MRS)	(8-57, MRS)	Open-label comparative study	Sitagliptin vs. Glimepiride	24 week	BMI did not change at any time for either treatment group, reduction in liver fat and total body fat following sitagliptin treatment was significant (-16.3% and -4.0%, respectively) but not significant with glimepiride (-28.9% and +6.6%, respectively)
Tang	2015	Type 2 diabetes	35	13.8% (MRI-PDFF)	(0-27.7)	Prospective, open-label, randomized	Insulin glargine vs liraglutide	12 week	Body weight was changed in the liraglutide group (-3.2%), insulin glargine treatment reduced liver fat using MRI-PDFF (-23.2%), liver volume (-7.6%), and total liver fat index (-31.2%), liraglutide exposure values were not significantly changes from baseline
Matthews	2015	Human immunodeficiency virus subjects with chronic hepatitis C infection	13	>5% (MRS), confirmed with biopsy	(0.0-31.2)	Double blind placebo controlled	Pioglitazone	48 week	Liver fat content in the pioglitazone treatment arm decreased from baseline (-49.7%, MRS, confirmed by biopsy)
Cui	2016	Pre-diabetics or early diabetics with NAFLD	41	17.4% (MRI-PDFF)	(0-42.3)	Randomized, double-blind, allocation-concealed, placebo-controlled	Sitagliptin	24 week	Group mean differences following sitagliptin treatment were no better than control in reducing liver fat (-6.6% vs. -15.7%, respectively), confirmed on MRS (-12.8% vs. -14.6%, respectively) and MRE (+3.8% vs. -3.6%, respectively)

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at 1 month (-28.4%), 6 months (-52.8%), months (-36.5%) and 1 year (-55.3%). Liver fat levels were reduced at 6 months (-57.4%) and 1 year (-69.7%) while the subcutaneous fat depot was reduced at 1 month (-10.1%), 6 months (-36.5%) and 1 year (-87.2%) accompanied by a liver

volume reduction at 6 months (-21.8%) and 1 year (-29.9%). The extramyocellular lipid levels were reduced at 6 months (-30.6%) and 1 year (-39.0%); however, the intramyocellular lipid levels were not different from baseline (6 months (+18.9%) and 1 year (-8.1%)). Post-surgery energy restriction, altered gut peptide secretion, and resumption of insulin sensitivity alter the amounts of lipids stored in several body adipose depots, especially in the liver where the greatest reduction was observed.

Since obese subjects may have difficulties fitting into cylindrical MRI systems, experiencing squeezing or claustrophobia, van Werven et al. [39] investigated the feasibility of using an open-system 1T 1H MR to assess fatty liver in morbidly obese subjects undergoing the same Roux-en-Y gastric bypass surgical procedure. The group correlated their baseline MRS liver fat values against histologic assessments, producing an accuracy of 89% (sensitivity 85%, specificity 94%, positive predictive value 94% and negative predictive value 83%). Liver fat decreased significantly from 5.8% (median, range 0.1-21.4%) to 3.1% (median, range -7.5-27.2%) in patients returning 3 months after surgery (-53.7%). The physical size on obese subjects is a serious consideration for selecting clinical trial sites. While open-bore systems may not have the signal-to-noise ratio seen in 1.5T or 3T MRI, large subjects that might otherwise have been excluded were able to be successfully imaged.

Calvo et al. [40] followed 19 obese NAFLD subjects who underwent bariatric surgery following the laparoscopic sleeve gastrectomy at 12 months, assessing liver fat along with an extensive panel of metabolic parameters. BMI and liver fat were significantly reduced (-28.9% and -80.0%, respectively) from baseline with a significant improvement in NAFLD staging by histology. It should be noted that while all subjects were obese, the baseline and 12 months liver fat levels were variable, suggesting that NAFLD was not a universal finding. While this group developed a liver-specific metabolomics platform, the results of the MRI fatty liver analysis alone was adequate to monitor subject response, suggesting that repeat histology and MRS metabolite analysis contribute marginally to NAFLD or NASH studies.

Studying a similar population of morbidly

obese women, but using a laparoscopic adjustable gastric banding surgical intervention, Heath et al. [22] tracked the metabolic and physical changes in 18 subjects at 3 and 12 months post-surgery. They used MRI to measure adipose tissue volume like the visceral and abdominal subcutaneous depots and MRS to measure liver fat. In this study, there were significant reductions in body weight at 3 months (-9.3%) and 1 year (-19.0%), significant reductions in visceral fat depot at 3 months (-21.7%) and 1 year (-37.1%), and significant reductions in subcutaneous fat depot at 3 months (-14.4%) and 1 year (-29.1%). The liver fat levels were reduced at 3 months (-36.4%) and 1 year (-45.5%), but these changes were not significant. Only 7 of the 18 women at baseline had levels that could be defined as fatty liver. The lack of a significant change in liver fat over time is in contrast to the Johansson et al. [20] report, but may relate to low pre-operative liver fat stores and/or to high post-operative dietary fat intakes [22].

Gastric bypass surgical intervention clinical trials are often small (average trial size ~20 subjects), self-controlled, and focused on a morbidly obese population. While we would expect to see high hepatic fat fractions at baseline, the screening ranges of 0.4% to 55% suggest that morbid obesity does not always equate to a high hepatic fat fraction. One point that is clear in this population is that the magnitude of liver fat reduction post-surgery is quite striking as early as 3 months (-41.5% average) and continues to decrease out to one year (-70.9% average).

■ Diet and exercise

Obesity is a risk factor in developing NAFLD. Thomas et al. [15] hypothesized that by restricting caloric intake in addition to starting a 10k steps a day exercise regimen over a self-controlled 6 month period, the combination of nutritional counselling and activity would have a beneficial effect on body weight, regional fat depots and intrahepatic and intramuscular lipid levels. These lifestyle modifications in the studied 10 subjects resulted in significant losses in body weight, total adipose tissue content (largest decrease in visceral adipose, smallest in the subcutaneous internal), waist circumference, serum HbA1c and tibialis intramyocellular lipid content. Regarding the liver fat, seven subjects had reductions while three experienced

increases from their pre-diet levels, making it difficult to draw any consistent conclusions regarding diet change and liver fat levels. Even with 3 increased cases, the group mean over the 6 month lifestyle change was -39.8% (MRS, range -69.5 to +18.4%). Four of the subjects had liver biopsies and the responders showed mild steatohepatitis whereas the non-responders showed progressive signs of severe inflammation and fibrosis, suggesting that fibrosis indicates a more advanced state of progression, and that more significant weight loss may be required to generate a reversal in liver fibrosis.

Abdominal obesity and fat depot distribution and metabolic activity are related to insulin resistance and fatty liver levels. Thamer et al. [17] performed a natural history cross-sectional analysis of 112 healthy, non-diabetic subjects with insulin sensitivity with type 2 diabetes risk factors such as abdominal obesity. Their analysis centered on determining relationships between subcutaneous/abdominal and liver fat depots using MRI and MRS methods, respectively. All subjects received pre-study counselling and started an exercise and dietary lifestyle intervention with monthly follow-up sessions, as part of the Tuebingen Lifestyle Intervention Study program. Data from the subject's final visit at ~ 9 months (\pm 60 days) showed that BMI, body weight, % subcutaneous fat, % visceral fat and liver fat were reduced from baseline. With changes in body fat levels and distribution, insulin sensitivity also increased, confirming that visceral adiposity and elevated liver fat amounts are associated with reduced insulin sensitivity independent from each other. However, subjects with high visceral fat mass or high liver fat at baseline did not achieve the same level of improved insulin sensitivity compared with leaner cohort members.

Johnson et al. [23] investigated if a short-term, 4-week aerobic exercise training program compared to a stretching control could alter blood, intramyocellular, abdominal (visceral and subcutaneous), and liver fat content. Obese adults ($n=23$) with a BMI ≥ 30 kg/m² reported to have a sedentary lifestyle with low alcohol intake (0-20 g/day) were screened/randomized, with 19 completing the study for analysis. Body weight, BMI, macronutrient composition and energy intake did not change from baseline. Visceral adipose tissue fat levels were significantly reduced in the

exercise group (-11.6%) compared to those performing stretching (+1.1%); subcutaneous adipose tissue fat levels were not different in the exercise group (-2.0%) compared to those performing stretching (-2.0%). Liver fat levels were significantly reduced in the exercise group (-20.6%) compared to those performing stretching (+2.8%). These results suggest that regular, short-term, aerobic exercise training can reduce visceral adipose tissue depot and liver lipids without concurrent changes in body weight or abdominal subcutaneous adipose tissue content.

Fatty liver is not limited to adults. Children with high BMI may also have concurrent fatty liver [41]. In a study of twenty-six 6 to 14 year olds, Pozzato et al. [30] over a one-year period investigated if a nutrition-behavior intervention consisting of normocaloric balanced diet and exercise would reduce fatty liver using MRI. This is the first treatment-related report using a 2 pt. Dixon in-phase/out-phase method. Modification to diet and exercise over the trial period resulted in significant reductions in BMI Z score (-12.7%) and waist circumference (-1.7%). Energy intake and nutrient analysis showed lower intakes of energy, total fat, saturated, monounsaturated, polyunsaturated fat, and carbohydrate, without significant difference in protein intake at the end of the intervention. The individual liver fat fraction had a wide variance, with the highest liver fat level at baseline being 36%. Of the nine children with the highest liver fat at baseline, seven of them had liver fat levels below the inclusion requirement of $\geq 9\%$, with this subgroup mean liver fat decreasing from 18.7% to 1.3%. This high liver fat subgroup displayed a liver fat reduction of -93.0% with the group mean reducing liver fat levels from baseline -63.5%. In addition to demonstrating that an exercise and diet modification can reduce body mass and liver fat, this study showed that MR imaging of liver fat in children was possible when a short image acquisition time (approximately 1.5 min) was employed.

Critically evaluating Hispanic adolescents, van der Heijden et al. [24] recruited groups of obese and lean boys and girls for a physical fitness intervention without an accompanying diet modification. These subjects started a monitored cardiovascular fitness exercise program, with the intention of studying the

visceral, subcutaneous, intramuscular and liver fat depots changes in otherwise healthy, but sedentary, individuals. Weight loss was not an objective and did not change significantly, but there was a small but significant decrease in overall fat mass in the obese group. Total energy intake and macronutrient distribution were virtually identical between groups over the 12 week study period. In the fat compartments, visceral and liver fat within groups was reduced in obese but not lean subjects -9.1% and -37.1%, respectively, over the exercise period with differences between groups. The subcutaneous fat and intramyocellular fat depots were not different within or between groups at baseline or 12 weeks. The large amount of liver fat change was driven by five subjects, with reductions ranging from 26-64%. Clinical trials designed to investigate a treatment effect often look at group means and percentage changes within and between groups. In small trials, individuals with large changes can drive these group means and contribute to group heterogeneity. It is important to evaluate these outliers, as their variation or response may not adequately predict a population's response.

Physical fitness programs may achieve better compliance if supervised; therefore, Cuthbertson et al. [28] monitored NAFLD subjects randomized to exercise supervision (n=30) versus counselling (n=20, both without dietary modification) for a period of 16 weeks, assessing changes in liver, subcutaneous, and visceral adipose fat depots. Median liver fat measured by MRS at the end of the study was significantly reduced in the exercise group (-47.9%) but not the counselling group (-8.8%), with a significant absolute difference between group means (-4.7%). Subcutaneous and visceral adipose tissue depot mean changes were significant in the exercise (-10.4% and -12.2%, respectively) but not the counselling group (+6.5% and +2.6%, respectively) with significant difference in group means for subcutaneous adipose (-10.4%) but not visceral adipose (+7.5%) depots. The reduction in liver fat with exercise was accompanied by an increase in insulin sensitivity in adipose fat depots and skeletal muscle.

Looking specifically at the effect of fatty acid composition in the diet, Bjermo et al. [38] used MRI and MRS to assess fatty liver changes in 67 abdominally obese subjects. In this controlled

trial, subjects were randomly assigned to a 10-week isocaloric diet which modified the fats to be either saturated fatty acid-based (i.e., butter, SFA) or vegetable fatty acid-based (i.e., n-6 polyunsaturated fatty acids, such as sunflower oil rich in linoleic acid or margarine, PUFA). MRI (3-pt. Dixon) and MRS were used to assess subcutaneous and visceral adipose tissue volumes and liver fat. From baseline, the energy intake and body weight increased slightly over the course of the study, but there were no differences between groups in either parameter. Liver fat values at baseline were not different between the SFA diet (7.5% and 3.2%, MRI and MRS, respectively) or PUFA diet (6.8% and 3.2%, MRI and MRS, respectively). Following diet modification, liver fat was significantly reduced during the PUFA diet (-7.4% and -28.1%, MRI and MRS, respectively) than during the SFA diet (0.7% and 0.3%, MRI and MRS, respectively) resulting in group mean differences of +9.3% and +9.4%, MRI and MRS, respectively. This is the first treatment-related report demonstrating fatty liver values using two different MRI methods. While these data indicate that a 3 pt. Dixon method produced a liver fat value approximately twice that observed using MRS, the percent differences are basically the same. Investigators who compare published results therefore need to consider methodology when looking at absolute values and should consider comparing percent differences in drawing conclusions about the magnitude of change.

Argo et al. [42] studied the n-3 PUFA found in fish oil in subjects (n=33) with non-cirrhotic NASH in a randomized, double-blind, placebo-controlled trial of 1 year duration. Both groups received counselling on nutrition/caloric intake and physical activity. The purpose of this study was to assess the histologic change from screening biopsy when adjusted for age and weight change. Fatty liver was also measured by 2-pt. Dixon MRI. Two different hepatopathologists reviewed the core biopsy results and concluded that the NASH activity score, fibrosis stages, and hepatocellular ballooning did not differ between treatment groups. Subjects in the n-3 fish oil treatment group had reduced liver fat and but not abdominal fat (-54.3% and -25.8%, respectively) while counselling alone did not reduce liver fat or abdominal fat (+6.2% and -12.3%, respectively) as measured using the 2-pt. Dixon MRI technique. Subjects taking

the n-3 fish oil did have a reduced liver fat as measured by MRI, but this observation was not seen as an overall improvement in biopsy results, suggesting a possible sensitivity to change superiority with MRI.

Docosahexaenoic acid is a long-chain, n-3 PUFA commonly found in fatty fishes, reported to have positive effects on hypertension, atherosclerosis, and inflammation. Pacifico et al. [33] investigated if docosahexaenoic acid found in algae oil supplements had a beneficial effect on liver, abdominal, and epicardial fat adipose depots following a 6 month intervention coupled with diet and exercise. Since the short-chain n-3 linoleic acid is poorly converted into docosahexaenoic acid, it was chosen as the control supplement. A double-blind, parallel-group, placebo-controlled randomized trial in 51 children with NAFLD confirmed by biopsy were measured with 3-pt. Dixon MRI at baseline and follow-up in 3 different regions of the liver along with visceral, subcutaneous, and epicardial adipose tissues. Docosahexaenoic acid treatment significantly reduced liver fat (-53.6%) with no significant change in placebo (-22.6%); visceral adipose fat depot was significantly reduced from placebo (-7.8% and -2.2%, respectively), and epicardial adipose fat depot was significantly reduced from placebo (-14.9% and -1.7%, respectively), but not subcutaneous adipose tissue depots (-3.8% and -1.6%, respectively). For feasibility and ethical reasons, a post-treatment biopsy was not performed.

In addition to diet and aerobic exercise, the effect that resistance training has on fatty liver change has been studied. Bacchi et al. [31] studied 40 sedentary type 2 diabetics in a randomized, controlled study of aerobic conditioning versus weight machine-based resistance training. All subjects received nutritional counselling for at least 2 months before commencing their group's activity for 4 months. The MRI 2-pt. Dixon method was used to assess visceral, superficial, and deep subcutaneous abdominal adipose tissue depots and liver fat content; DXA was used for total body fat mass analysis. Total daily caloric intake remained the same in both groups for the duration of the study. BMI, total body fat mass, visceral, subcutaneous, superficial subcutaneous and deep subcutaneous abdominal adipose tissue depots were reduced from their respective baseline with no difference between treatment groups at the 4-month assessment

(MRI). Both exercise regimens elicited a comparable, marked, absolute, and relative reduction in liver fat content from baseline (-37.7% aerobic, -38.2% resistance). Changes from baseline but not between treatment groups were found in visceral (-30.3% aerobic, -19.0% resistance), subcutaneous (-9.2% aerobic, -12.4% resistance), superficial subcutaneous (-13.1% aerobic, -17.1% resistance), and deep subcutaneous (-4.7% aerobic, -3.8% resistance) abdominal adipose tissue depots. Both treatments improved insulin sensitivity. At the end of the study 7 of the 30 subjects had a liver fat less than 5.56%, a commonly used threshold for declaring a liver fatty [43,44].

Nutritional supplements containing bioactive compounds may be beneficial in treating NAFLD and NASH. Chachay et al. [26] designed a small randomized, double-blind, placebo-controlled trial in 20 NAFLD overweight or obese men exposed for 8 weeks to Japanese knotweed (*Polygonum cuspidatum*)-derived resveratrol or placebo. They used MRI to assess changes in visceral and subcutaneous abdominal adipose depots, with liver fat analysis using MRS. While resveratrol was well-tolerated and its administration did not promote fat oxidation, exposure failed to alter any of the fat depots. Neither group had a change in body weight, visceral (-2.3% resveratrol vs. -6.5% placebo) or subcutaneous (+1.4% resveratrol vs. +2.1% placebo) adipose depots, or fatty liver (+8.0% resveratrol vs. -3.8% placebo). Alanine (ALT) and aspartate (AST) aminotransferases increased linearly among subjects in the resveratrol vs. placebo group until week 6 (ALT, +55% resveratrol vs. +8% placebo; AST +50% resveratrol vs. -3% placebo) when both marker values returned to ~20% higher than baseline in the resveratrol group, but not differ from placebo (ALT, 13%, AST -6%). Scaling the resveratrol dose from mouse to human did not produce the desired effect, since it failed to promote fatty acid oxidation, reduce body weight, increase sensitivity to insulin, reduce inflammation, or promote antioxidant activity.

Many of the fatty liver investigations published start with subjects that are overweight or obese with an elevated liver fat liability. Dietary modifications have the potential to alter liver fat levels and Agebratt et al. [29] studied 30 non-obese subjects for two months looking at diets with increased fruits or nuts (diets were

isocaloric at 7 kcal/kg bodyweight/day). MRI measures included liver fat content, along with visceral and subcutaneous adipose depots at screening and follow-up. Weight gain and BMI increases occurred in both groups, but only the changes in the nut group were weakly significant (+0.9%, $p=0.049$ BW, +0.8%, $p=0.049$ BMI). The increases in the nut group were attributed to increased calories tabulated from food diaries (+9.7%). In these modified diets, daily fruit consumption (g/day) increased (+181.3%) while nut consumption decreased (-47.6%) but liver, subcutaneous, or visceral adipose depots were unchanged. It should be noted that these subjects, at the start of the study, had the lowest liver fat levels of those in this review at 2.09% as measured by MRI-PDFF, and may serve as a good reference when benchmarking what is a 'normal' liver level.

As with many interventions, the sustainability or durability of the response is the true measure of a good clinical outcome. Subjects who lose weight are no exception, and dieting subjects eventually return to their historical eating habits sedentary lifestyle. Haufe et al. [25] revisited a dieting study cohort at an average of 24 months after their initial diet trial, to investigate if the losses in body weight, abdominal fat depot, and intrahepatic fat remained. Of the initial 108 overweight and obese subjects, 50 were available for this long-term follow-up analysis. The baseline and 6 month data values were previously collected before similar tests were performed. The range of the follow-up window was 17-36 months. Body weight was reduced at the 6 month visit (-7.9%) but returned to only -4.9% of baseline, a significant increase from the 6 month mean (+3.2%), but still significantly lower than baseline. Subjects were stratified according to NAFLD status at baseline. The diet intervention resulted in a significant decrease at 6 months with rebounding during the long-term follow-up in visceral (-20.3% No NAFLD, -20.3% with NAFLD at 6 months, -15.1% No NAFLD, -6.6% with NAFLD at follow-up) and subcutaneous (-16.8% No NAFLD, -15.1% with NAFLD at 6 months, -7.4% No NAFLD, -7.5% with NAFLD at follow-up) abdominal fat masses. While there was a rebound in this follow-up window, the values remained significantly reduced from baseline. In contrast, liver fat decreased during the dietary intervention and remained reduced at follow-

up (-35.5% No NAFLD, -44.3% with NAFLD at 6 months, -16.1% No NAFLD, -49.1% with NAFLD at follow-up), with no difference between the 6 month and follow-up fatty liver values. All improvements displayed some durability of response, despite regain of body weight. While abdominal visceral adipose tissue and subcutaneous adipose tissue mass increased during follow-up, the lowered liver fat was sustained during the follow-up time window.

Lifestyle changes represent the least invasive way to modify hepatic fat fraction (and other fatty depot) values. By changing your diet and increasing your activity with exercise, it is fair to assume that a beneficial outcome would be a reduction in fat values. In several studies, total body weight loss and specific adipose depot reductions in addition to hepatic fat fraction were achieved. In the 13 randomized prospective trials we identified, the average study enrolled 42 subjects from a variety of subject populations. Liver fat was reduced in 10 of 13 trials, with lifestyle modification producing an average reduction of ~ 41%. It should be noted that the time taken to produce this effect varied from 10 weeks to 1 year.

Drug Investigations

■ Thiazolidinedione class, peroxisome proliferator-activated receptor γ agonists/ligands

Thiazolidinedione anti-diabetic drugs are orally active agents that improve insulin action. Expressed predominantly in adipose tissue, when the nuclear peroxisome proliferator-activated receptor γ (PPAR γ) is occupied, lipogenic genes are expressed, resulting in adipocyte growth and differentiation. Carey et al. [14] investigated rosiglitazone exposure on insulin sensitivity and regional adiposity in patients with type 2 diabetes in a 16-week double blind placebo controlled trial. MRS was used to measure right lobe liver fat and lumbar MRI was used to measure subcutaneous and visceral fat compartments. With a treatment window of 16 weeks, total body weight and fat mass increased. Rosiglitazone exposure increased subcutaneous fat volume (+8.2%), did not change the visceral fat volume (+1.5%) and significantly reduced liver fat levels (-30.5%). Controls had no significant changes from baseline in subcutaneous fat volume (+1.6%),

visceral fat volume (+1.4%), or liver fat levels (+2.6%). While body weight can serve as a general indicator of fat burden, rosiglitazone exposure can result in what appears to be paradoxical or contradictory changes (e.g. body weight increased while liver fat levels decreased).

Promrat et al. [45] reported the first thiazolidinedione clinical trial specifically in NASH subjects. Using biopsy histology to stage subjects for fatty liver, lobular inflammation, and zone 3 ballooning degeneration, 18 subjects started a 3 month pre-treatment evaluation designed to lose weight, stop taking over-the-counter vitamins and supplements and follow a healthy diet before commencing a 48 week course of oral pioglitazone. Each subject served as his or her own control. At the end of treatment, histologic fatty liver, parenchymal inflammation, and hepatocellular injury improved in all subjects with many seeing improvements in fibrosis scores and Mallory bodies. A biochemical analysis of serum samples showed no apparent correlation between adipokine levels at the start of therapy and liver biopsy NASH histological scores, adiponectin level nor leptin level correlated well with histology or MRI fatty liver, and liver fibrosis did not correlate significantly with serum levels of proinflammatory cytokines, leptin or adiponectin [46]. Patients experienced weight gain during pioglitazone therapy, with DXA showing an increase in adiposity and total fat mass with no change in lean body mass. MRI measurements of liver fat (+47.5% pre-treatment, +22.8% at Week 48) and liver volume (2,276 ml pre-treatment, 1,963 ml at Week 48) decreased significantly. This early NASH study with liver fat measured by MRI and correlated with histologic changes begins to develop a biomarker foundation where MRI liver fat can be used as a surrogate for histologic change.

Zib et al. [18] studied obese subjects with type 2 diabetes, investigating if pioglitazone added to an insulin regimen reduced triglyceride levels in the liver (and heart), as well as the subcutaneous and visceral fat masses. In this 32 subject prospective, randomized, open-label study, subjects gained weight (significant increases from baseline: +9.4% after pioglitazone plus insulin treatment, +6.1% in the insulin-only comparator group, but not different between treatment arms, as measured by MRS);

subcutaneous fat mass increased (significant increases from baseline: +20.4% after pioglitazone plus insulin treatment, +22.2% in the insulin-only comparator group, but not different between treatment arms); visceral fat mass remained unchanged (+11.6% after pioglitazone plus insulin treatment, +7.3% in the insulin-only comparator group); and the average liver fat content significantly decreased after pioglitazone plus insulin treatment (-52.1%) with the insulin-only comparator group decreasing -24.1%. In a similar type 2 diabetes subject population, Ravikumar et al. [21] investigated 10 subjects on sulfonylurea therapy, adding in pioglitazone for a treatment period of 16 weeks. Their analysis of visceral and liver fat was like that of Zib et al. pioglitazone treatment was associated with significant increases in body weight (+7.1%) and subcutaneous fat (+15.7%) with accompanying significant decreases in visceral fat-to-subcutaneous fat ratio (-16.2%, note: total visceral fat content was unchanged) and liver fat content decrease of -52.2%. These results show that thiazolidinedione agents exert insulin-sensitizing effects on fat and skeletal muscle, reducing cardiac and liver fat levels, even in the face of overall body weight gain and adipose redistribution.

In subjects with human immunodeficiency virus (HIV), often a co-infection with chronic hepatitis C virus (HCV) exists. These comorbid HIV/HCV subjects are at risk of developing fatty liver, fibrosis and in the worst case, hepatocellular carcinoma. Mathews et al. [27] investigated a small set of HIV/HCV subjects to determine if pioglitazone treatment would reduce liver fat measured by MRS. In this 48-week double blind placebo controlled 13 subject investigation, pioglitazone treatment significantly decreased liver fat content as measured by MRS by -49.7% from baseline (confirmed by biopsy) while a non-significant reduction of -15.7% was observed in the placebo group. The between-group difference was not significant, possibly due to between-subject variability and small sample size. Methods studies and drug pilot studies are often underpowered, and in studies with a small sample size and paired analyses, subject phenotypic heterogeneity at enrolment along with compliance and retention should be considered when assessing variability or addressing the impact of missing data.

Eriksson et al. [19] reported the results from the Mechanisms for the Diabetes Preventing Effect of Candesartan Study (MEDICA), a multicenter 3-way crossover trial with 22 non-diabetics, abdominally obese and hypertensive completers designed to assess insulin action/secretion and body fat distribution after treatment with candesartan, hydrochlorothiazide, and placebo. Looking at adipose tissue distribution in the abdomen, liver and anterior tibial muscle when subjects alternated treatment for a 12 week window, receiving each treatment in a double-blind manner, the analyses demonstrated that subject drop-out was high, only hydrochlorothiazide treatment reduced body weight (-1 kg, $p < 0.01$ vs. placebo). Candesartan had no significant differences in indices of abdominal obesity or in the percentage of body fat. Median MRS liver fat content following crossover was ranked as hydrochlorothiazide (+6.6%) > candesartan (+4.8%) \approx placebo (+3.9%), with hydrochlorothiazide demonstrating statistical differences from both candesartan and placebo. The increase in liver fat with hydrochlorothiazide compared with placebo significantly correlated with the reduction in insulin sensitivity. No significant differences in the amount of intramyocellular and extramyocellular lipids were found between treatments. The subcutaneous adipose tissue:visceral adipose tissue ratio was significantly lower only after hydrochlorothiazide treatment. These data support that hydrochlorothiazide treatment can produce visceral fat redistribution, liver fat accumulation, aggravated insulin resistance, and low-grade inflammation in abdominally obese hypertensive subjects.

Not all thiazolidinedione class intervention studies were designed to investigate a reduction in liver fat. In a study by Arioglu et al. [47], lipotrophic diabetic subjects were recruited to assess if 6 month troglitazone exposure could help achieve glycemic exposure while increasing fat mass. Using DXA and MRI, these low adipose subjects demonstrated an increase in total body fat (+2.4% absolute increase, DXA) while liver volume decreased (-12.5%, MRI). Increases in regional subcutaneous (+19.2%) but not visceral fat (-16.7%) were also observed with MRI (body weight was unchanged). While not specifically looking at liver fat levels, fractional oxidation of fat was an important

molecular pathway to achieving their desired outcome. Body composition can be measured successfully using DXA and MRI, with MRI adding volumetric analysis to the list of study biomarkers.

■ Leptin

With fatty liver occurring due to a decrease in leptin action, Javor et al. [48] investigated 10 subjects with generalized lipodystrophy (8 patients) or Dunnigan's partial lipodystrophy (2 patients) in a self-controlled study of recombinant methionyl human leptin (r-metHuLeptin) administration. Liver biopsies and MRI of the liver were acquired at baseline and during treatment (mean treatment period, 6.6 months). r-metHuLeptin exposure resulted in significant reductions in liver volumes (-25.5%), liver fat (-64.5%), transaminases, triglycerides, fasting glucose, fasting insulin and hemoglobin A1c levels. Significant reductions in NASH scores were appreciated on follow-up biopsy, specifically in scores for fatty liver, ballooning injury, and parenchymal inflammation. Interestingly, there was no change in fibrosis score, and 3 subjects had a slight or mild amount of residual fatty liver. The results demonstrated in this small study without a placebo group that correlation between the liver biopsy NASH activity scores and MRI estimations of liver volume and fat content exist.

■ Insulin

In type 2 diabetics, it is unclear whether fat in the liver is the cause or the consequence of the hyperinsulinemia. Juurinen et al. [16] investigated whether basal insulin therapy changed body composition, liver fat content or liver insulin sensitivity in 14 type 2 diabetes subjects poorly controlled by metformin. After 7 months of daily insulin, these subjects displayed a significant increase in body weight (+3.0%), no change in subcutaneous (+1.9%) or visceral (+0.0%) fat mass, and a significant reduction in liver fat content (-17.6%). The results demonstrate that a significant correlation between changes in liver fat and liver insulin sensitivity during chronic insulin therapy. Since liver fat content decreased, the metabolic alterations favoring inhibition of lipogenesis or other alterations resulting in reduced liver triglyceride stores must have dominated. In this study, insulin therapy increased liver insulin

sensitivity and slightly decreases liver fat content in obese type 2 diabetic patients, suggesting that hyperinsulinemia that accompanies a fatty liver is a consequence rather than cause.

Supporting the positive role that supplemental insulin can have on type 2 diabetics, Tang et al. [36] evaluated the effects of insulin glargine versus liraglutide (a Glucagon-Like Peptide-1 Receptor [GLP-1] Agonist) therapy in 35 subjects, randomized to receive one of the treatments for 12 week, with liver fat and volume measured with MRI-PDFF and MRS. Insulin glargine treatment trended toward decreasing liver fat by MRS (-21.4%) and achieved significance when measured using MRI-PDFF (-23.2%), liver volume (-7.6%) and total liver fat index (-31.2%). The same parameters following liraglutide exposure were not significantly changed from baseline. Body weight was not changed with insulin glargine treatment (+0.0%) but was reduced in the liraglutide group (-3.2%), with a developing trend between weight loss and reduction in MRI-PDFF. In this investigation MRI-PDFF was more sensitive to changes in liver fat than single voxel MRS methods.

■ Cholesterol and bile acid disruptors

Colesevelam is a bile acid sequestrant that interrupts bile acid enterohepatic circulation and results in an increased conversion of liver cholesterol to bile acids. Since colesevelam exposure produces a glucagon-like-peptide-1 (GLP-1) level increase, stimulating pancreatic beta cells to release insulin. Le et al. [34] investigated if colesevelam would decrease liver fat in patients with biopsy-proven non-alcoholic steatohepatitis, measuring liver fat with MRI-PDFF and MRS techniques. A total of 77 study candidates were screened to enroll 50 subjects, 25 subjects in each treatment arm. This was a randomized, double-blind, allocation-concealed, placebo-controlled 24 week trial. Unexpectedly, colesevelam treatment increased liver fat (+19.7% and +15.3%, MRI-PDFF and MRS, respectively) compared to placebo liver fat measures (-15.1% and -14.4%, MRI-PDFF and MRS, respectively). In those subjects with biopsies and liver histology, no difference was detected at the microscopic level, and histology supported the small increase in fatty liver in the treatment versus control arm. Histology has always been considered the

overall gold standard for NASH assessment, but with its limited tissue sample size, may not accurately convey the distribution or severity across the entire liver. MRS up to this point in the literature is considered the imaging gold standard. What this investigation begins to show is that the use of MRI-PDFF as a sensitive and alternative method, especially when assessing at the Couinaud segment level, suggests its measurement equivalence and validity is possible, warranting further treatment trial investigation.

Secondary analyses to the Le et al. study above centered on the effect of weight loss on liver volume, fat fraction along with total liver fat burden or index [49]. Patel et al. [50] analyzed data from the colesevelam trial, partitioning the subjects into those who did (n=10) or did not (n=33) have at least a 5% decrease in BMI. The subjects with significant weight loss demonstrated a relative decrease in liver fat (-25.5%) and decrease in liver volume (-5.3%), both of which were significant. Tang et al. [49] calculated that the product of the MRI-PDFF and liver volume (with the units %·mL) effectively integrates the fatty liver content and its respective volume into a total liver fat index. In their analysis, this index was significantly increased +22.9% with colesevelam treatment, a decreased trend in the placebo group (-16.8%), with a significant overall group effective difference (-18.4%) between groups. Since BMI changes of this magnitude can drive changes in liver fat and liver volume, drug intervention trials where both BMI and liver fat may change will require careful interpretation to avoid attributing the treatment effect to drug exposure, dismissing the confounding effect that weight loss could have in producing the reduction in liver fat.

Ezetimibe inhibits the absorption of cholesterol from the small intestine. By changing the dynamics at the level of the hepatocyte, cholesterol is removed from circulation by increased hepatocellular uptake. Attempting to translate the results from a rat study to NASH humans, Loomba et al. [35] evaluated ezetimibe exposure in reducing MRI-PDFF while adding in MR elastography endpoints. Inclusion required $\geq 5\%$ MRI-PDFF as well as steatosis on historical biopsy. A total of 50 subjects were randomized to 24 weeks of either ezetimibe or

placebo. At the conclusion, MRS was performed to confirm the MRI-PDFP analysis. Liver fat analysis was performed by Couinaud segment and group means were generated. There was a significant MRI-PDFP difference in the ezetimibe group from baseline (-22.7%) but not from the placebo group (-11.4%), confirmed by MRS (-22.3% and -9.9%, respectively; $r^2=0.95-0.99$). Ezetimibe exposure did not result in histological changes, although on an individual basis, five subjects in each arm did experience a 2-point reduction in their NAFLD activity score. Those experiencing a histological response had a significant reduction in liver fat level (-4.35%) compared to non-responders (+0.30%). MR elastography, whether measured with 2D or 3D methods, did not result in any treatment-related changes in liver fibrosis. The changes in liver fat quantified by MRI-PDFP were confirmed by MRS and supported with histology, helping to validate MRI-PDFP as an alternative to these gold standard assessments. Large-scale studies across diverse populations are needed to further validate a role of MRI-PDFP as a suitable way to assess liver fat changes in clinical trials.

■ Dipeptidyl peptidase-4 inhibitor class

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released in response to meals. A new class of oral antihyperglycemic known as dipeptidyl peptidase-4 (DPP-4) inhibitors function to inhibit the enzyme(s) which inactivate GLP-1 and GIP. This mechanistic dis-inhibition increases insulin secretion and suppresses glucagon release from the pancreas. This novel mechanism was investigated by Cui et al. [37] in a population of pre-diabetic or early diabetics with NAFLD. In a 50-subject randomized, double-blind, allocation-concealed, placebo-controlled trial, sitagliptin was tested to reduce liver fat levels in 24 weeks, using MRI-PDFP, confirmed by MRS, with an exploratory endpoint of MR Elastography to assess liver fibrosis. On average, group mean differences following sitagliptin treatment were no better than control in reducing liver fat, -6.6% vs. -15.7%, respectively, which was confirmed on MRS using co-localized regions of interest over the Couinaud segments (-12.8% vs. -14.6%, respectively). Changes in liver stiffness values measured by MR Elastography also were not different (+3.8% vs. -3.6%, respectively). MRI-PDFP and MRS displayed significant correlation

coefficients ($r=0.96-0.99$). With confidence in our image methods, NASH studies in the future may move away from liver biopsies an inclusion requirement or as a significant efficacy endpoint.

Type 2 diabetics are often initially treated with metformin in addition to lifestyle modification such as diet and exercise, with a goal of achieving glycemic control and HbA1c reductions. Kato et al. [12] investigated a small population ($n=20$) of overweight Japanese subjects with type 2 diabetes who were fatty liver-positive on ultrasound exam at eligibility, randomized to an open-label course of sitagliptin or glimepiride, a sulfonylureal. Liver fat was assessed by MRS with total body fat measured by DXA. Results suggested that BMI did not change at any time for either treatment group, reduction in liver fat and total body fat following sitagliptin treatment was significant (-16.3% and -4.0%, respectively) but not significant with glimepiride (-28.9% and +6.6%, respectively). Variability in liver fat measurements was higher following treatment in the glimepiride group (10.2-39.0%) compared with the sitagliptin group (14.6-28.5%), influencing these statistics. While both treatments result in improved glycemic control, only sitagliptin exposure resulted in the added reduction of liver fat.

Other Consideration

While technically not a surgical procedure, venipuncture is a common procedure during a drug intervention trial, and the amount, method, and frequency of repeated blood draws could alter iron level acutely or chronically or cause subjects to drop-out from the study. In an interesting methods study, Adams et al. [32] suggested that since iron plays a role in insulin resistance and liver injury, they hypothesized that phlebotomy could be a treatment in NAFLD, reducing fatty liver. Screening 104 confirmed fatty liver subjects and randomizing 74 into prospective venesection with lifestyle advice versus lifestyle advice alone groups, 61 subjects remained in the study at the end of six months. Subjects in the venesection group underwent phlebotomy every 2-3 weeks, with removal of 250-300 ml per session. Both groups received counselling about exercise and diet advice on reducing fat as part of a low-calorie diet. Liver assessments included a 3-pt. Dixon MRI analysis with iron concentration derived using the FerriScan service (Resonance Health,

Claremont, AUS). Phlebotomy did not change body weight, serum alanine aminotransaminase, fatty liver (-5.2% phlebotomy vs. -10.9% counselling alone) or iron levels (-32.6% phlebotomy vs. -9.1% counselling alone) from baseline to 6 months. The authors conclude that iron removal via venesection was not associated with an improvement in fatty liver, liver injury, insulin resistance, lipid peroxidation or quality of life. While these might be viewed as negative results, for those performing longitudinal clinical trials with multiple blood draws, these are reassuring data that the repeated venipuncture procedures are not introducing any untoward impacts to certain serum or tissue-based measurements.

Pharmaceutical intervention trials (n=16) using MRI methods to assess changes in hepatic fat fraction have a varied design (from open-label to double-blind, placebo-controlled) and tend to be small, with an average trial size ~25 subjects. The study populations tend to focus on areas of unmet medical needs, such as fatty liver in type 2 diabetes (n=6) or NAFLD/NASH (n=6). Dosing duration is often in the 12-24 week range, and the efficacy results are often variable. The overall average liver fat at baseline was ~16% (facilitated by studies where sufficient hepatic fat presence was confirmed by biopsy) with the low end of the enrollment being ~6.5% hepatic fat fraction as measured by MRI, in alignment with the designation of MRI steatosis as measured in excess of 6%, suggesting a good starting point for minimum subject eligibility amount required in future trials.

Discussion

This review illustrates that relative liver fat at eligibility varies based on the subject population and methodology, ranging from ~2% in non-obese individuals to ~31% in those with generalized lipodystrophy. Ranges in liver fat at screening create statistical issues and opportunities: excluding low liver fat subjects while enrolling subjects with higher levels of liver fat may make it easier to detect a reduction over time, showing that similar populations at baseline demonstrate a treatment effect using a repeated measures design (i.e., each subject in a treatment group serves as its own control). Small sample sizes are common in single-site trials, and with the exception of Eriksson et al. [19], the trials reviewed here represent published

single-site investigations. As we embark on multi-center trials, MR instrumentation from multiple vendors may be used, suggesting that reproducibility and smallest detectable differences be assessed whenever possible to reduce any scanner bias or excessive variability.

In an effort to be able to compare values across studies, we converted some of the results to percentages. These calculations derived from numerical values contained within individual reports or were estimated from figures. Ranges calculated from standard deviations or standard errors of the mean attempt to estimate a 95% confidence interval when actual ranges were not reported. We did not, however, perform any analyses comparing trials, which would make for an interesting future meta-analysis.

Fatty liver is a constant feature of NASH, but NASH is only distinguishable by liver biopsy. Not all trials included biopsies, and some of the trials with biopsies experienced difficulties in obtaining multiple or repeat biopsies. Some of the reasons for no repeat biopsy included subject withdrawal of consent, development of a biopsy contraindication (such as abnormal PT/PTT) during treatment, pediatric subjects, and exclusion of patients after baseline biopsy established the diagnosis of a liver disease other than fatty liver. We often view the histology from biopsies as being confirmatory, yet with improvements in MR methods, it appears that MR liver in some studies was equivalent or potentially superior in sensitivity to histopathologic scoring for liver fat. In studies which included histology with MRI liver fat analyses, the superior anatomic coverage of MR techniques generates confidence in MRI as a surrogate for histologic cellular change.

This review of clinical trials with MR fatty liver endpoints concentrated on the subject populations, their baseline fatty liver level, and their respective baseline ranges of liver fat to demonstrate expected variability. Determining an inclusion criterion on the amount of fat needs to be considered in NAFLD and NASH trials, since this parameter is critical in designing study objectives and reasonable estimates of response, given the length of intervention. Not all drugs in development are at a stage that permits 6 months of dosing, and being able to identify early signs of efficacy might be critical in selecting candidates for future larger trials. Alternatively, no signs of

efficacy may be an important stopping point for a candidate unlikely to be successful in a larger, longer multisite trial that is surely to be more expensive than a pilot investigation.

We did not include methods development papers, but acknowledge their importance in establishing the clinical utility of the endpoint and its readiness for use in clinical trials. Readers are directed to other pre-clinical [51],

methods development [52,53], reproducibility [13,54] or clinical utility [55] reports for further information.

This extensive review of lifestyle and pharmacological trials which evaluated liver fat levels changes using MR techniques will serve as a useful reference for those developing trials particularly in NAFLD and NASH subjects.

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