Effects of Controlled Hypocaloric Ketogenic and Low-Fat Diets on Liver Fat in Overweight/Obese Adults

THESIS

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Abstract

Background & Aims: The ketogenic diet (KD) has been shown to be effective at reversing manifestations of insulin resistance including non-alcoholic fatty liver disease (NAFLD). Whether KDs have an advantage over low-fat diets to decrease liver fat when matched for energy under controlled conditions remains unclear, as does the impact of exogenous ketone supplementation. The primary objective of this study was to compare composite liver fat responses to a precisely controlled hypocaloric ketogenic diet, with and without an exogenous ketone supplement, versus an energy-matched low-fat diet in overweight/obese adults.

Approach and Results: Adults with excess weight and variable liver fat at baseline (mean 4.9%, range 0.5 to 23.0) were assigned to precisely controlled hypocaloric (75% of energy expenditure) 6 week diets that were ketogenic (KD+PL), ketogenic with ketone salt supplementation (KD+KS), or low fat (LFD). Weight loss was significant ($P<0.001$) but similar between groups (6.7 to 8.5% of initial body mass, $p > 0.05$). Composite liver fat assessed by magnetic resonance imaging decreased significantly ($p=0.004$) but was similar across KD+KS (-42%), KD+PL (-32%), and LFD (-52%) groups ($p > 0.05$). In the subset of individuals with NAFLD (liver fat >5%), the decreases in liver was of greater magnitude, but similar between the KD groups (-56%, n=7) and LFD (-60%, n=5). There were changes in serum albumin and alkaline

phosphatase concentrations over time but no differences between groups. Neither AST nor ALT responses were significant. **Conclusions:** These results indicate that short-term hypocaloric KDs decrease liver fat independent of supplementation with exogenous ketones and to a similar extent as an energy-matched low-fat/high-carbohydrate diet.

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Chapter 1. Introduction

Excessive adiposity affects a third of the world population with projections half of people globally will be obese by 2030 (GBD, 2015; Finkelstein et al., 2012). Obesity is strongly associated with multiple common diseases such as cardiovascular disease, type 2 diabetes, hypertension, and non-alcoholic fatty liver disease (NAFLD) (Formiguera & Canton, 2012). NAFLD is a progressive liver disease characterized by increased accumulation of fat in the liver (>5% fat), that if left untreated, can lead to more advanced forms of liver disease such as non-alcoholic steatohepatitis, fibrosis, cirrhosis, and liver failure (Must & Strauss, 1999). The exact mechanisms involved in NAFLD remain unclear but it involves an imbalance between factors that contribute to delivery of fat to the liver (i.e., dietary fat intake, release of fatty acids from adipose tissue, and *de novo* lipogenesis) with pathways involved in hepatic fat disposal (i.e., fatty acid oxidation, ketogenesis, VLDL triglyceride secretion) (Stefan et al., 2008; Mardinoglu et al., 2018; Gaggini et al., 2013). The multi-factorial regulation of these regulatory factors is complex but become biased toward accumulation of hepatic fat in people who have insulin resistance (Gastaldelli et al., 2007; Mishra et al., 2008; Donnelly et al., 2005). Since pharmacological interventions are of limited benefit, the preferred treatment for NAFLD are diet interventions promoting weight loss (Romero-Gómez et al., 2017; Fan & Cao., 2013). Weight loss by caloric restriction is helpful in reducing liver fat storage by imposing energy deficits that the body must meet by utilizing stored fat as endogenous fuel. But, whether the macronutrient distribution of

hypocaloric diets affects NAFLD differentially remains unclear. This is due in part because of challenges associated with proper formulation and accurate control of energy in dietary interventions.

From a dietary perspective, low-fat/high-carbohydrate diets could be effective in treating NAFLD because they limit dietary intake of fat, but the impact on hepatic fat disposal may not favor net loss of fat due to increased DNL and reduced fatty acid oxidation and/or ketogenesis. In contrast, low-carbohydrate ketogenic diets (KDs) inherently contain higher fat, but they significantly increase whole body fatty acid oxidation and hepatic ketogenesis which may offset increased availability and delivery of fat to the liver (Mardinoglu et al., 2018; Donnelly et al., 2005; Luukkonen et al., 2020). Thus, despite vastly differential effects on regulatory factors impacting liver fat, both low-fat/high-carbohydrate and high-fat/low-carbohydrate diets could hypothetically reduce liver fat (Yki-Järvinen., 2015; Haufe et al., 2011; Tendler et al., 2007; LaFountain et al., 2019; Hyde et al., 2019; Kirk et al., 2009), but whether one diet has an advantage is controversial. Hypocaloric KDs rapidly decrease liver fat after just a few days (Mardinoglu et al., 2018; Luukkonen et al., 2020; Kirk et al., 2009), but have similar effects to a low-fat diet after the first few months (Kirk et al., 2009). Similarly, a hypocaloric low-carbohydrate and low-fat diet were both equally effective at decreasing liver fat after 6 months (Haufe et al., 2011), but another recent study showed a more favorable effect of KD that was likely due to greater caloric restriction (Cunha et al., 2020).

Whether a KD uniquely benefits liver fat compared to other diets remains unclear due to study limitations, notably a lack of controlled feeding and formulation of ketogenic diets. Not all low-carbohydrate diets are ketogenic, and therefore may not maximally decrease liver fat since

partitioning of hepatic fatty acids into ketogenesis is a major mechanism by which KDs rapidly decrease liver fat (Luukkonen et al., 2020). The lack of controlled feeding and matching of energy between diets, unknown dietary compliance/adherence, proper KD formulation, and detailed monitoring of ketosis are limitations in previous KD work. Many studies also do not report information on regional changes in liver fat or liver enzymes, which are commonly used for NAFLD referral.

In order to address limitations in previous KD studies assessing effects on liver fat, we performed a controlled feeding study with precisely defined hypocaloric ketogenic and low-fat diets over a 6-week period in obese subjects. An additional element that could impact the response to a KD is provision of exogenous ketones that augment circulating concentrations of ketones. Exogenous ketones have been theorized as beneficial for NAFLD pathology, possibly even without the KD, for its inducement of ketosis (Watanabe et al., 2020). Since ketones inhibit adipose tissue lipolysis (Stubbs et al., 2017), they could positively impact fat balance by decreasing hepatic fatty acid delivery. Thus, we also included a diet group to explore the effects of supplementing a KD with an exogenous ketone salt supplement on liver fat. The primary outcome was composite liver fat assessed by MRI, as well as individual liver segments to address potential regional differences in liver fat response (Idilman et al., 2013). Secondary outcomes included changes in liver enzymes and other markers associated with liver health.

Chapter 2. Literature Review

Obesity is becoming a pandemic across the world, affecting developed and developing countries alike. By 2030, projections indicate approximately half of the world will classify as overweight or obese (GBD 2015; Finkelstein et al., 2012). Obesity carries with it significant disease risk, including high mortality rate, life changing diseases such as Metabolic Syndrome and its many comorbidities (Global BMIM et al., 2016). One of these is non-alcoholic fatty liver disease (NAFLD).

2.1 NAFLD

NAFLD is the most prevalent liver disease in the world and currently affects about a third of the population in North America (Fazel et al., 2016). This disease is characterized by increased fat storage in the liver, with the threshold for NAFLD set at 5% liver fat fraction in individuals who are not alcoholics. This disease is progressive, with increased fat storage eventually leading to inflammation of hepatic cells, or non-alcoholic steatohepatitis (NASH), that eventually scar and become fibrotic tissue (cirrhosis) (Ali and Cusi 2009). Cirrhosis can cause death and is only treatable by liver transplant. There is an urgent need for better methods of detection and prevention of NASH during its asymptomatic early stage before cellular damage occurs (Fazel et al., 2016).

Methods of Assessing Liver Fat

Current clinical diagnosis of NAFLD hinges upon elevation of liver functional enzymes, specifically alanine and aspartate aminotransferase (ALT and AST, respectively). Following that, the prospective patient is recommended for liver biopsy, the current clinical gold standard test for evaluating hepatic fat histological fibrosis. However, it is not uncommon for NAFLD patients to have completely normal liver enzyme levels in even the most advanced cases of the disease (Fazel et al., 2016; Clark et al., 2003). In addition, liver biopsy is invasive, can have complications, and is suboptimal for early diagnosis due its small sample size, cost, and clear difficulty with repeated measurement necessary to track disease progression (Hines et al., 2011).

Due to these difficulties, a number of advanced imaging methods have been proposed as alternatives. Advanced imaging, in general, offers advantages over biopsy of being non-invasive and therefore better for longitudinal follow-up (Schwenzer et al., 2009). Non-invasive alternatives include ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) as well as spectroscopy (MRS) (Schwenzer et al., 2009). Each of these imaging modalities have their own respective strengths and weaknesses. Ultrasound has become a popular NAFLD screening tool due to low-cost and clinical availability (Ricci et al., 1997). Ultrasound has noted drawbacks of poor measurement sensitivity and reproducibility (Debonginie et al., 1981; Kinner et al., 2016), an overreliance on operator subjectivity (Strauss et al., 2007), along with an inability to differentiate steatosis from fibrosis (Taylor et al., 1981; Kinner et al., 2016). CT has been used as well. CT is reliable and accurate for whole liver fat quantification (Schwenzer et al., 2009). However, CT has had limited use clinically and for research because results differ based on manufacturer (Birnbaum et al., 2007), it is inaccurate in

non-severe cases of NAFLD (Park et al., 2006), and CT exposes the patient or subject to radiation. MRI and MRS are both accurate, reliable, and are able to quantify fat, as well as fibrosis, regionally and across the whole liver leading to MR being seen as a potential one-stop measure of NAFLD progression (Kinner et al., 2016). In comparison, MRS may be more accurate than MRI due its direct assessment of fat and water spectroscopy, but faces a downside in that it is typically only collected in a single region of interest, similar to liver biopsy (Dulai et al., 2016). Furthermore, both MR modalities offer differing sequences and techniques, each with their upsides and downsides. In general, both MR techniques have limited uses in patients with bodily implants, claustrophobia, or in people unable to perform long breath-holds; additionally, both techniques offer practical constraints such as device and scan cost in addition to a lack clinical availability, particularly in the case of MRS (Schwenzer et al., 2009). Despite new imaging techniques, diagnosis of NAFLD remains difficult due to its complex pathophysiology and its multi-factorial mode of development.

NAFLD Pathophysiology: The Multiple Hit Hypothesis

The pathophysiology of NAFLD is not completely understood and develops due to a variety of factors. At its most basic level, NAFLD develops when the liver stores fat past a healthy threshold and the liver becomes histologically diseased as NAFLD progresses. The liver and its relationship with fat exist in a balanced state in a normal, healthy individual; there is no net gain or loss of liver fat, over an appreciable time, as the healthy person equally delivers and disposes fat to and from the liver, creating a neutral fat flux with storage comprising <5% of tissue. But, an imbalance skewing the liver fat balance toward the delivery of fat to the liver at the expense of disposal from the liver, results in increasing amounts of fat deposition and the

origin of a diseased state. The mechanism by which this occurs is referred to as the multiple hit hypothesis (Polyzos et al., 2009; Buzzetti et al., 2016).

The multiple hit hypothesis by Buzzeti et al. describes that this imbalance in fat handling comes via “hits” from multiple factors. These hits cascade into a furthered diseased phenotype. This first cascade is the development of obesity and insulin resistance, predisposed by poor diet, sedentary lifestyle, and an unfortunate genotype (Romero-Gómez et al., 2017). Insulin resistance creates whole-scale dysfunction in the physiological handling of fats leading to increased DNL and release of fatty acids from adipose tissue, as well as inhibited lipolysis resulting in a net increase of fat delivery to the liver (Gaggini et al., 2013). Consequently, fat storage increases within the liver. Within the scope of early stage NAFLD and simple steatosis, this storage is relatively benign (Buzzetti et al., 2016). But, this increased storage, combined with other “hits” and epigenetic factors, can lead to chronic hepatic inflammation, cellular dysfunction or death, and fibrosis, progressing the disease and worsening patient outcome.

A number of treatments have been put forth, including vitamin E, pharmaceuticals, and lifestyle interventions with diet and exercise (Musso et al., 2012; Romero-Gómez et al., 2017). Of these potential treatments, dietary and exercise interventions have had the most success as pharmaceuticals have not proven effective (Romero-Gómez et al., 2017). This has led to further research into nutrition as a preventative measure and treatment for NAFLD. One specific diet in particular has been posited as being physiologically beneficial: the ketogenic diet (KD).

2.2 The Ketogenic Diet

The KD is characterized by consumption of low carbohydrate, moderate protein, and high fat, food intake. The crucial distinction between a KD versus a low carbohydrate diet is that a KD is <50g carbohydrate daily. The metabolic state of ketosis results from habitual consumption of KD, marked by blood ketone concentration being greater than 0.5 mmol/L. In ketosis the body readily switches fuel preference from glucose to fats, thereby increasing whole body fatty acid oxidation and hepatic ketogenesis (Donnelly et al., 2005; Mardinoglu et al., 2018). Despite higher intake of dietary fat, increased liver fat outflow has led consideration of KD as a potential treatment and preventative measure for NAFLD and other metabolic diseases.

Ketogenic Dietary Interventions on Liver Fat

Several studies have investigated the effects of the KD on liver fat storage and health in a variety of diet formulations, caloric restrictions, control groups, and study designs. There are major differences in effects seen depending on caloric restriction and studied population. LaFountain et al examined the effects of a KD versus a mixed diet (MD) in a healthy military population with *ad libitum* feeding, and daily fasted ketone and glucose measurements to ensure adherence. Both the KD and MD groups had healthy, <5% liver fat at baseline. While the KD group lost significantly more body weight than the MD group, neither diet caused any significant change in liver fat percentage (LaFountain et al., 2019). Lafountain et al. is the first study to quantify liver fat in healthy individuals and they found in non-NAFLD healthy populations, there is no overall, average response at the group level. Accordingly, due to there being so little research of fat changes when the subject is already below NALFD criteria, there is no literature

on whether or not there is an associated health benefit when losing liver fat when already below 5%.

Liver fat reductions have not been seen as significant in diseased populations on isocaloric diets in one study by Hyde et al. A balanced cross-over study design utilizing a variety of carbohydrate levels (low, moderate, and high) in isocaloric diets in obese metabolic syndrome patients (n = 16) measured liver fat percentage using MRI (Hyde et al., 2019). 15 of the 16 patients classified as NAFLD. Although the diets were isocaloric and no body composition changes were seen, the liver fat averages for each diet were lower than the baseline liver fat average (13.9%). There was a trend for lower liver fat percentage from the low carbohydrate/KD compared to the moderate and high carbohydrate diets (p = 0.072). Due to the BMI of these obese patients, some of these patients ate >300 g/day of fat. This massive intake of fat likely offset the reduced DNL and enhanced fatty acid delivery. Unfortunately, although these results are promising, no other studies have investigated the effects of isocaloric diets of differing composition on NAFLD populations.

The level of caloric restriction was seen to also affect the magnitude of liver fat lost in one study by Cunha et al. In a comparison of a hypocaloric (15% energy restriction) mixed diet to a very low calorie (800 kcal/day) KD, the very low calorie KD reduced liver fat to greater extent (-47%) compared to the mixed diet (-8.8%) (Cunha et al., 2020). Interestingly, these changes in weight and BMI correlated weakly with liver fat % changes.

NAFLD populations utilizing hypocaloric diets and the KD have seen significant improvements in liver fat percentage and overall liver health during acute interventions

(Luukkonen et al., 2020; Sevastianova et al., 2011, Kirk et al., 2009). Short term hypocaloric KD have resulted in 30-40% reductions in liver fat percentage in interventions lasting up to six days (Luukkonen et al., 2020; Sevastianova et al., 2011, Kirk et al., 2009). These acute reductions in liver fat have not extended to liver enzyme improvements however, and have remained unchanged with the exception of AST/ALT, which worsened. (Kirk et al., 2009; Luukkonen et al., 2020).

Short to moderate term (weeks to months) hypocaloric KD interventions have shown consistent improvements in all facets of liver health and show potential as a possible treatment of NAFLD (Tendler et al., 2006; Haufe et al., 2011). Six month weight loss interventions have shown histological improvements in steatosis, inflammatory grade, and fibrosis, as well as in serum cholesterol and bilirubin (Tendler et al., 2006). Hepatic fat was reduced following a six-month hypocaloric diet as measured by magnetic resonance spectroscopy and tomography (Haufe et al., 2011).

The KD has been shown to be effective in improving liver health during dietary weight loss interventions, but its effects have rarely been compared to other diets while controlled for caloric intake. There have been two studies that compared a hypocaloric KD to a hypocaloric low-fat diet (Haufe et al., 2011; Kirk et al., 2009). KD have a stronger acute effect on liver fat reduction than do low-fat diets after a period of 48 hours (Kirk et al., 2009). But, when the interventions extend for months, it has been demonstrated in previous studies that weight loss, liver fat reductions, and liver enzyme responses are similar between the two diets (Haufe et al., 2011; Kirk et al., 2009).

KD have proven to be as effective as low-fat diets for improving liver health during short term hypocaloric dietary interventions. In as little as two months both diets are capable of eliciting statistically, and clinically, significant losses of liver fat. However, the literature base contains many limitations when taken as a whole. Some major limitations are lack of controlled feeding for both diets, diet formulation of a KD as opposed to low-carbohydrate, energy matching, unknown compliance and adherence, detailed monitoring of ketosis, lack of liver enzyme reporting, and no evaluation of regional fat changes within the liver. The current study is a three arm, six week, hypocaloric controlled feeding dietary intervention measuring liver effects from a ketogenic diet with placebo (KD+PL), a ketogenic diet with ketone salt supplement (KD+KS), and a control low fat diet (LF) in an obese population. This study was designed to examine the effect macronutrient composition has on the liver in a more controlled study hypocaloric dietary intervention, while also examining the effect of elevated concentrations of circulating ketones from ketone salt supplementation.

Chapter 3. Methods

Study Design

The study design was a three-arm prospective controlled feeding intervention for 6 weeks in overweight/obese men and women. The diet interventions were a ketogenic diet with ketone supplement (KD+KS), ketogenic diet receiving a placebo (KD+PL), and a low-fat diet receiving the same placebo (LFD). Twenty-seven participants were matched for age and BMI and then randomly assigned in a double-blind manner into either the KD+PL or KD+KS groups. After completion of the ketogenic diet arms of the study, a separate group of twelve participants, also matched for age and BMI, were recruited for the LFD. This was done in part due to practical difficulties of buying ingredients and cooking for two separate diets with different menus, and also due to this study being a part of a larger overarching trial.

Participants

Participants were overweight and obese men (*n = 18*) and women (*n = 19*), as determined by BMI (27-35kg/m2), between the ages of 21-65 years with a weight loss goal to ensure adherence to a hypocaloric diet. Exclusion criteria included: >10% weight loss in prior six months, endocrine dysfunction, smoking, drug use, alcoholism, epilepsy, headaches, pregnancy, or use of antibiotic medication, or current use of a ketogenic diet. Screening meetings were

scheduled for participants who qualified where initial eligibility assessments were performed including a food frequency survey, medical history, physical activity questionnaire, MRI screening, and menstrual history. Eligible participants signed an informed consent document approved by the Ohio State IRB. There were no significant differences between groups in baseline characteristics (**Table 1**).

Table 1

Participant Characteristics

	KD+KS (n=12)	KD+PL (n=13)	LFD (n=12)	p-value
Sex (male/female)	6/6	6/7	6/6	
Age (years)	35 ± 3	35 ± 3	35 ± 3	0.99
Weight (kg)	90.4 ± 3.4	94.1 ± 3.2	92.4 ± 3.4	0.73
BMI (kg/m2)	30.6 ± 0.7	31.8 ± 0.7	30.9 ± 0.7	0.50
Capillary Ketones (mmol/L βHB)	0.18 ± 0.03	0.18 ± 0.04	0.13 ± 0.02	0.40

Values reported as mean ± SEM. p-value obtained from one-way ANOVA.
BMI = body mass index; VAT = visceral adipose tissue; βHB = beta-hydroxybutyrate

Diet Intervention

This was a controlled hypocaloric feeding study where all food was prepared in a metabolic kitchen. Each daily meal was prepared and provided to the participants with strict instruction to eat nothing else. Dietitians and research staff collaborated to develop meal plans and prepare appetizing meals to ensure the highest possible compliance and adherence. Each ingredient was precisely weighed (±0.1g) with custom macro- and micronutrient composition personalized to each participant using advanced nutrient analysis software (Nutritionist Pro, Axxya Systems, Redmond, WA). Both KD groups were designed based on previous well-formulated standards (Hyde et al., 2019) while the LFD was developed according to USDA Dietary Guidelines for Americans 2015-2020 (USDA, 2015). Participant menus were calculated from a base caloric level of 2,000 kcal and scaled to match 75% of the individual participants

estimated energy expenditure based on information obtained from resting energy expenditure using indirect calorimetery, a standard Harris-Benedict equation for estimating energy expenditure, and the energy cost of their physical activity. Protein was set at 1.5 g/kg reference weight for all three diets, a portion of which was provided as twice daily protein shakes containing whey protein isolate (~15g/serving) and fat containing high oleic sunflower oil and medium chain triglycerides (MCTs). Finally, subjects in both KD arms consumed a serving of MCT oil (caprylic and capric acid) with their breakfast and afternoon snacks. Average daily nutrient intake over the 6 weeks for each group is shown in **Table 2**. A particular note of difference between the diets is sodium intake. This significant difference is due to the twice daily consumption of the ketone salt in the KD + KS group.

Table 2

Diet Composition

	KD+KS	KD+PL	LFD
Energy (kcal)	1845 ± 102	1752 ± 98	1900 ± 102
Protein (g)	99 ± 3	100 ± 3	100 ± 3
Carbohydrate (g)	40 ± 8[a]	38 ± 7[a]	259 ± 8[b]
Sugar (g)	17 ± 3[a]	17 ± 3[a]	101 ± 3[b]
Fiber (g)	10 ± 1[a]	10 ± 1[a]	34 ± 1[b]
Added Sugars (g)	n/a	n/a	<25g/day
Fat (g)	143 ± 9[a]	131 ± 8[a]	51 ± 9[b]
SFA (g)	63 ± 4[a]	63 ± 4[a]	17 ± 4[b]
MUFA (g)	38 ± 3[a]	38 ± 3[a]	10 ± 3[b]
PUFA (g)	8 ± 1	8 ± 1	7 ± 1
Cholesterol (g)	414 ± 27[a]	402 ± 26[a]	154 ± 27[b]
Sodium (mg)	6100 ± 32[a]	2351 ± 30[b]	1974 ± 31[c]
Potassium (mg)	2211 ± 73[a]	2243 ± 75[a]	2758 ± 78[b]
Calcium (mg)	2001 ± 36[a]	880 ± 34[b]	1008 ± 35[c]

Values reported as mean ± SEM. Distinct letters denote group differences (p<0.05)

The KD+KS group consumed a ketone supplement twice daily consisting of βHB salts and noncaloric flavoring (Metagenics). One serving contained 11.8 g βHB, 1874 mg sodium, 570 mg calcium, and 57 mg magnesium. βHB content was determined to contain a racemic βHB enantiomer mixture of R- βHB and S- βHB. Participants were instructed to mix the ketone salts with at least eight oz of water and stir. The KD+PL group and LFD groups received a calorie-free and flavored placebo containing no βHB or minerals. It was identical in taste and appearance to protect the double-blind nature of the study for the KD groups.

Testing Battery

All participants reported biweekly to the testing facility between 5:00 and 7:00am for a three hour testing battery at baseline, wk-2, wk-4, and wk-6.. Participants were instructed to arrive having consumed no caffeine >12 hours or food >6 hours, be in a rested state (8-10 hours of sleep) and having refrained from exercise the prior two days. Urine specific gravity was measured using a light refractometer to ensure hydration (>1.025). If determined dehydrated, the participant was required to drink 8 oz of water until achieving euhydration.

The testing battery consisted of body composition measures, resting metabolic rate (RMR), plasma blood draw, capillary finger sticks, and supplement ingestion. Body composition included height and weight and a DXA (GE Lunar DXA, Madison, WI) scan determining whole body composition and bone density conducted by a certified DXA technician. RMR was measured with indirect calorimetry (ParvoMedics TrueOne 2400) in a dark, quiet, and temperature controlled room. A hood was placed over the subject's head to collect samples of air

from inhalation and exhalation while breathing normally. Subjects rested in a supine position for 20 min, then gas exchange was measured for 25 min in 15 sec intervals. The value reflective of daily RMR and respiratory exchange ratio to determine substrate use were selected from a stable, average RMR recorded from final 5 min of continuous readings to obtain steady state readings. Plasma blood was drawn from an arm vein by a qualified phlebotomist. Ketone and glucose capillary measures were done every 20 min after supplement ingestion during the testing visit, and then hourly after leaving the lab. Each participant also took daily morning finger sticks while fasted.

MRI Acquisition

Each participant was imaged once at baseline and after the six week diet intervention on a 3T MRI scanner (MAGNETOM Prisma Fit, Siemens Healthineers, Erlangen, Germany) at the same location (Martha Morehouse Medical Plaza at 2050 Kenny Rd, Columbus, OH 43221). Abdominal liver fat scans were completed in each session. The total duration of the testing session averaged 1h (**Table 3**).

Table 3

MRI Sequence Parameters

	Field Strength (T)	Total Slice (#)	Acquisition Time (s)	Slice Thickness (mm)	Slice Coverage (mm)	FOV_x (mm)	FOV_y (mm)
Fat Imaging (Abdominal)	3 Tesla	64	18	4.8	307	282-390	418-500

The VARiable PROjection (VARPRO) pulse sequence was used to acquire the in-phase, out-of-phase, water, water percentage, fat, and fat percentage images that were used to measure liver fat fraction. The VARPRO pulse sequence is a single breath hold acquisition that collects the multiple echo time images required for fat/water separation (Hernando et al., 2008). This rapid scan technique acquires 3D volumetric images covering the entire abdominal region in a single breath-hold to acquire abdominal and liver fat.

Hepatic Fat Quantification

A single operator with 5 years of cardiac and fat MRI quantification experience performed all analysis. Hepatic fat percentage was quantified using a previously described MRI technique that has become standardized in its objective quantification of proton density fat fraction (Springer, Claussen, & Schick, 2010). For liver fat, the fat fraction was measured in each of the nine segments of the liver by manually placing circular ROIs in three slices defined by the anatomy. First, the hepatic portal vein (HPV) slice was defined as the slice separating the five superior segments from the 4 inferior segments, and the top half from bottom half of the liver. Then, the most superior and most inferior slices in which each liver segment was visible and free from significant artifact or vasculature obstructions were identified and used for quantification. ImageJ software (Rasband, 2012) was used to draw circular ROIs (17.32 mm diameter) in all segments at the most superior or inferior slice, the slice nearest the HPV, and the slice in between and equidistant to both, avoiding large blood vessels and visible image artifacts. Thus, 3 ROIs were drawn in each of the 9 liver segments. The 3 measurements in each segment were

averaged over the height of the liver to measure segmental fat fraction, and then the measurements for the 9 segments averaged to provide a single liver fat fraction for each subject.

Statistical Analysis

All analysis was completed using SPSS. Results featuring the full sample of the three groups was completed using a 3x2 repeated measures ANOVA using a general linear model. Results revolving around the NAFLD subgroup were analyzed using a 2x2 repeated measures ANOVA with a general linear model. All correlation data was calculated using bivariate Pearson correlations.

Chapter 4: Results

Weight Loss and Liver Fat Responses

Mean weight loss after 6-weeks was significant ($p<0.001$) but similar between diets representing 8.1, 8.5, and 6.7 percent of initial body mass in the KD+KS, KD+PL, and LFD groups, respectively ($p > 0.05$). Within each group there was variation of weight loss in response to the dietary intervention as the weight loss ranges were 9, 5.3, and 12 kg in the KD + KS, KD + PL, and LFD groups. As determined from whole body DXA, most of the weight loss was attributed to decreased fat mass, although a portion was derived from lean mass. There were no differences in changes in fat or lean mass between groups. There were no significant differences in liver fat at baseline between groups. There was a significant ($p=0.004$) decrease in liver fat post-intervention in the KD+KS (-42%), KD + PL (-32%), and LFD (-52%) groups (**Figure 1A**) but no group or interaction effects ($p > 0.05$).

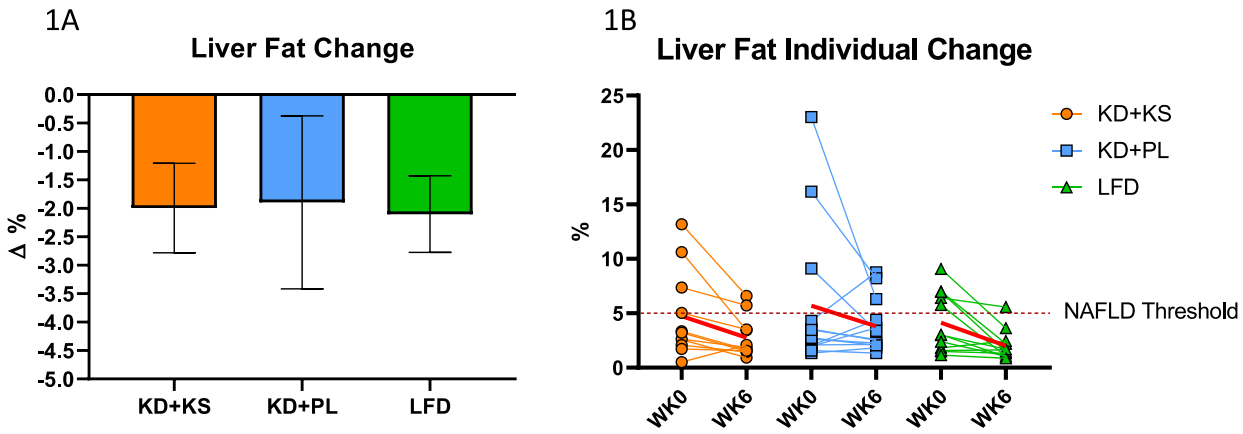

Figure 1: Liver fat change post-intervention by group (1A); Liver fat individual changes within group (1B).

Liver fat at baseline varied considerably between subjects across the groups including several who exceeded the 5% diagnostic threshold for NAFLD. Generally, subjects below 5% liver fat at baseline showed a variable response to dietary intervention, but those above 5% consistently decreased liver fat regardless of the magnitude of weight loss (**Figure 1B**). Thus, we performed a subgroup analysis in individuals with NAFLD (>5% liver fat), which included 4 participants from the KD+KS group, 3 from the KD+PL group and 5 from the LF group. The KD groups were combined due to low sample sizes and lack of difference between the two groups. Post-intervention the NAFLD subgroups decreased liver fat to a similar extent in the ketogenic combined group (-56%) and LFD (-60%) resulting in a significant reduction within group ($p = 0.001$). There were no differences between groups nor an interaction effect. All participants with NAFLD regardless of diet demonstrated a decrease in liver fat ranging from -13 to -82%. After the six week intervention 7 of the 12 subjects no longer met the NAFLD (3 from KD, and 4 from LF groups) diagnostic threshold of 5%.

Examination of the individual liver segments (1, 2, 3, 4a, 4b, 5, 6, 7, and 8) revealed a similar decrease as the composite liver fat (**Table 4**). There were no significant differences in liver fat between segments. Finally, there was also no difference of change between segments due to the intervention.

Table 4

Liver Lobes Fat Distribution

		Timepoint		3x2 ANOVA Effects		
Segment	Diet	WK0	WK6	Group	Time	Group*Time
Superior Lobes						
	KD+KS	4.75 ± 1.10	2.74 ± 0.41			
1	KD+PL	5.66 ± 1.56	3.69 ± 0.59	0.005*	0.16	0.73
	LFD	3.93 ± 0.70	1.96 ± 0.33			
	KD+KS	4.52 ± 1.10	2.91 ± 0.49			
2	KD+PL	5.73 ± 1.90	3.85 ± 0.64	0.016*	0.13	0.65
	LFD	3.67 ± 0.69	2.01 ± 0.20			
	KD+KS	4.93 ± 1.26	1.96 ± 0.42			
4a	KD+PL	5.53 ± 1.65	3.75 ± 0.73	0.001*	0.23	0.61
	LFD	3.95 ± 0.69	1.89 ± 0.49			
	KD+KS	5.21 ± 1.25	2.70 ± 0.68			
8	KD+PL	5.96 ± 2.01	4.13 ± 0.81	0.003*	0.33	0.82
	LFD	4.71 ± 0.93	2.16 ± 0.55			
	KD+KS	4.58 ± 1.58	3.44 ± 0.85			
7	KD+PL	5.76 ± 1.97	3.69 ± 0.75	0.009*	0.39	0.66
	LFD	4.22 ± 0.91	2.08 ± 0.44			
Inferior Lobes						
	KD+KS	4.68 ± 1.29	2.89 ± 0.62			
3	KD+PL	5.87 ± 1.79	3.73 ± 0.67	0.004*	0.19	0.71
	LFD	4.08 ± 0.74	2.08 ± 0.36			
	KD+KS	5.01 ± 1.43	2.42 ± 0.60			
4b	KD+PL	5.92 ± 1.91	3.74 ± 0.64	0.005*	0.43	0.88
	LFD	4.83 ± 1.07	2.06 ± 0.52			
	KD+KS	4.71 ± 1.04	2.65 ± 0.70			
5	KD+PL	5.42 ± 2.08	3.93 ± 0.89	0.011*	0.26	0.78
	LFD	4.16 ± 0.95	1.99 ± 0.59			
	KD+KS	4.34 ± 1.31	3.08 ± 0.89			
6	KD+PL	5.39 ± 1.85	3.70 ± 0.75	0.007*	0.31	0.80
	LFD	3.70 ± 0.76	1.75 ± 0.38			

*Values reported as mean ± SEM. * = p<0.05.*

Correlate Markers

Alkaline phosphatase significantly decreased after the intervention in each group (p-value < 0.001) while albumin significantly increased in both KD groups, but was unchanged in LFD (p-value = .04) (**Table 5**). Despite these differences, there were no significant group or interaction effects. There were no differences in other liver function enzymes: AST, ALT, AST/ALT, or bilirubin from baseline to post-intervention (p-values > 0.05). Fasting capillary ketones increased in all groups but the magnitude was greater in the KD + KS and KD + PL groups. This ketone increase was significant (P-value <0.001) and so were the group differences (p-value <0.001) between the KD groups and the LFD. Glucose decreased in both KD+KS and KD+PL groups, but remained unchanged in the LFD group. RER decreased in both KD+KS and KD+PL while staying the same in the LFD group. This resulted in significant time and group effects (p-value < 0.001).

Table 5

Serum Biomarkers and Anthropometry Changes

		Timepoint		3x2 ANOVA Effects		
Category	Diet	WK0	WK6	Time	Group	Group*Time
Serum Biomarkers						
AST (U/L)	KD+KS	19.7 ± 1.40	18.0 ± 1.63	0.32	0.26	0.7
	KD+PL	18.9 ± 1.67	18.8 ± 1.75			
	LFD	24.2 ± 1.75	21.3 ± 1.73			
ALT (U/L)	KD+KS	23.2 ± 3.30	21.3 ± 2.90	0.72	0.74	0.49
	KD+PL	21.0 ± 3.67	22.5 ± 3.41			
	LFD	28.3 ± 4.51	20.9 ± 2.06			
AST/ALT Ratio	KD+KS	0.96 ± 0.08	0.94 ± 0.08	0.51	0.3	0.4
	KD+PL	1.07 ± 0.11	0.96 ± 0.11			
	LFD	0.99 ± 0.09	1.10 ± 0.11			
HOMA-IR	KD+KS	2.58 ± 0.59	1.45 ± 0.26	.001*	0.45	0.99
	KD+PL	2.68 ± 0.58	1.42 ± 0.29			
	LFD	3.15 ± 0.39	1.96 ± 0.40			
Bilirubin (U/L)	KD+KS	0.54 ± 0.06	0.55 ± 0.06	0.39	0.45	0.54
	KD+PL	0.73 ± 0.16	0.71 ± 0.18			
	LFD	0.64 ± 0.07	0.78 ± 0.12			
ALP (U/L)	KD+KS	60.8 ± 3.52	48.4 ± 2.38	<0.001*	0.55	0.21
	KD+PL	63.3 ± 7.30	55.6 ± 4.64			
	LFD	62.7 ± 5.48	57.5 ± 5.37			
Albumin (mg/dL)	KD+KS	4.38 ± 0.06	4.49 ± 0.09	0.044*	0.16	0.15
	KD+PL	4.41 ± 0.09	4.59 ± 0.11			
	LFD	4.57 ± 0.09	4.55 ± 0.10			
Glucose (mg/dL)	KD+KS	94.0 ± 6.00	88.0 ± 3.0	0.035*	0.87	0.16
	KD+PL	99.0 ± 4.00	84.0 ± 4.0			
	LFD	92.0 ± 3.00	93.0 ± 2.0			
Ketones (mmol/L BHB)	KD+KS	0.18 ± 0.03	1.36 ± 0.28	<0.001*	<0.001*	<0.001*
	KD+PL	0.18 ± 0.04	1.12 ± 0.12			
	LFD	0.13 ± 0.02	0.21 ± 0.03			
Anthropometry						
Weight (kg)	KD+KS	90.4 ± 3.7	83.1 ± 3.2	<0.001*	0.46	0.21
	KD+PL	94.1 ± 2.8	86.1 ± 2.8			
	LFD	92.4 ± 3.4	86.3 ± 3.8			
DEXA FM (kg)	KD+KS	31.1 ± 1.5	26.4 ± 1.4	<0.001*	0.94	0.34
	KD+PL	34.5 ± 2.3	30.2 ± 2.2			
	LFD	33.4 ± 2.4	29.2 ± 2.5			
RER (VCO2/V02)	KD+KS	0.83 ± 0.01	0.77 ± 0.01	<0.001*	<0.001*	0.68
	KD+PL	0.86 ± 0.02	0.78 ± 0.01			
	LFD	0.88 ± 0.02	0.85 ± 0.01			

*Values reported as mean ± SEM. * = p<0.05.*

AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; HOMA-IR = HOMeostatic Assesment model of Insulin Resistance

Liver fat change scores from baseline to post-intervention were correlated with changes in body composition, liver function enzymes, ketones, and glucose within each group shown in **Table 6**. KD+PL significantly correlated with ALT (R= 0.57, p-value= 0.042), AST/ALT (R= -

0.0583, p-value = 0.037), and HOMA-IR (R= 0.763, p-value = 0.002). Meanwhile, in the LF group liver fat change significantly correlated with ALT (R= 0.676, p-value= 0.016), bilirubin (R= -0.86, p-value= 0.015), and alkaline phosphatase (R= 0.66, p-value= 0.02). The KD+KS liver fat changes from baseline to post-intervention did not significantly correlate with any liver functional markers or physiological measures. Furthermore, weight loss did not significantly correlate with any of the three diets (p-value > 0.05).

Table 6

Pearson Correlations between Liver Fat and Serum Biomarkers/Athropometric Changes

	KD+KS		KD+PL		LFD	
Category	R	P-value	R	P-value	R	P-value
Serum						
AST (U/L)	-0.17	0.63	0.37	0.21	0.36	0.25
ALT (U/L)	-0.03	0.93	0.57	0.042*	0.676	0.016*
AST/ALT	0.033	0.92	-0.583	0.037*	-0.406	0.19
HOMA-IR	0.031	0.93	0.763	0.002*	0.303	0.34
Bilirubin (U/L)	0.141	0.68	-0.072	0.82	-0.68	0.015*
ALP (U/L)	-0.41	0.21	0.065	0.83	0.66	0.02*
Albumin (mg/dL)	-0.118	0.73	0.46	0.11	-0.47	0.13
Glucose (mg/dL)	0.223	0.51	0.263	0.39	-0.175	0.59
Ketones (mmol/L BHB)	-0.538	0.09	-0.062	0.84	-0.221	0.49
Anthropometry						
Weight (kg)	0.28	0.40	0.041	0.89	0.079	0.81
MRI VAT (g)	0.23	0.50	-0.182	0.55	0.594	0.04
DEXA FM (kg)	0.544	0.08	0.003	0.99	0.037	0.91
RER	-0.075	0.82	0.085	0.78	0.352	0.26

*Values in bold face and * = p<0.05.*

The same correlate marker analysis performed for the 3 full groups was performed for the NAFLD subgroups as well. Of the liver enzymes analyzed above, each decreased in both the ketogenic combined group and the LF NAFLD subgroups to healthier levels, with the notable exception of albumin. ALP was the only enzyme to reach statistical time significance (p-value = 0.01), while AST reached significant group effects (p-value = 0.024). Other than those two

significant effects there were no other significant time, group, or interaction effects for the other analyzed enzymes (**Table 7)**. Meanwhile, glucose and ketone changes mirrored that of the whole sample (**Table 7**). Glucose decreased for the Ketogenic Combined subgroup, while glucose increased for the LF subgroup post-intervention. This did not result in time, group, or interaction effects (p-value > 0.05). Ketones increased precipitously in both the Ketogenic Combined and LFD NAFLD subgroups producing a significant time effect (p-value = 0.002), group effect (p-value = 0.02), and interaction effect (p-value = 0.004). Correlations were not performed in the subgroup due to small sample sizes.

Table 7

Serum Biomarkers and Anthropometry Changes - NAFLD Subgroup (KD n=7; LFD n=5)

		Timepoint		3x2 ANOVA Effects		
Category	Diet	WK0	WK6	Time	Group	Group*Time
Serum Biomarkers						
AST (U/L)	KD	21.4 ± 1.60	17.9 ± 1.87	0.1	0.024*	0.71
	LFD	27.6 ± 2.16	22.8 ± 1.93			
ALT (U/L)	KD	34.3 ± 4.68	24.0 ± 2.96	0.06	0.33	0.55
	LFD	40.4 ± 6.95	25.4 ± 2.93			
AST/ALT Ratio	KD	0.70 ± 0.12	0.77 ± 0.08	0.26	0.57	0.45
	LFD	0.73 ± 0.08	0.93 ± 0.11			
Bilirubin (U/L)	KD	0.51 ± 0.06	0.49 ± 0.06	0.16	0.1	0.06
	LFD	0.64 ± 0.07	0.98 ± 0.25			
ALP (U/L)	KD	61.4 ± 6.1	52.3 ± 3.60	0.01*	0.25	0.98
	LFD	76.6 ± 10.0	66.6 ± 10.7			
Albumin (mg/dL)	KD	4.44 ± 0.12	4.53 ± 0.07	0.53	0.42	0.86
	LFD	4.64 ± 0.18	4.70 ± 0.21			
Glucose (mg/dL)	KD	97.3 ± 6.2	84.7 ± 2.81	0.35	0.45	0.21
	LFD	84.8 ± 6.6	86.2 ± 3.34			
Ketones (mmol/L BHB)	KD	0.36 ± 0.12	1.59 ± 0.33	0.002*	0.02*	0.004*
	LFD	0.09 ± 0.01	0.17 ± 0.02			
Anthropometry						
Weight (kg)	KD	98.3 ± 3.6	89.9 ± 2.66	<0.001*	0.78	0.1
	LFD	94.4 ± 7.8	89.7 ± 8.84			
DEXA FM (kg)	KD	75.1 ± 3.8	65.0 ± 3.48	<0.001*	0.66	0.2
	LFD	79.3 ± 8.2	71.8 ± 9.02			

*Values reported as mean ± SEM. * = p<0.05.*

Chapter 5: Discussion

The primary objective of this study was to compare composite liver fat responses to a precisely controlled hypocaloric ketogenic diet, with and without an exogenous ketone supplement, versus an energy-matched low-fat diet in overweight/obese adults. The diets varied considerably in fat content with the ketogenic diet having nearly three-fold higher total fat and nearly four-fold higher saturated fat content than the LFD. In this context of short-term (i.e., 6-wk) variation in macronutrient distribution where the overall level of caloric deficit was standardized resulting in similar weight and fat loss, there was a similar decrease in liver fat independent of diet composition. This beneficial effect on composite liver fat was reflected across the various liver segments. We also examined for the first time whether a KD supplemented with exogenous ketones to specifically elevate BHB concentrations impacted liver fat responses. The results showed no additional impact of exogenous ketones on weight loss or liver fat. Our population included a mix of overweight individuals with variable liver fat. The most consistent improvements in liver fat were observed in those classified as having NAFLD (i.e., >5%). These results highlight the importance of achieving a consistent caloric deficit to decrease liver fat in the short-term (6 weeks), which could be achieved using a variety of different dietary approaches.

Liver fat reduction in hypocaloric dietary interventions comparing low fat and ketogenic diets are in line with prior studies in both magnitude and similarity in effect between diets when calorically matched (Haufe et al., 2011; Kirk et al., 2009). We believe this effect to be moderated by the controlled caloric restriction between diets, as other studies have seen differences in liver fat reductions with hypocaloric KD and low-fat diets unmatched in caloric restriction (Cunha et al., 2020). However, weight loss weakly correlated with liver fat change throughout the intervention, similar to what has been reported by others (Cunha et al., 2020). Although the decrease in liver fat occurred independent of diet composition, the underlying metabolic responses contributing to the net loss of hepatic fat varied considerably between the KD and low-fat diet in response to the imposed energy deficit. KD + PL and KD + KS consumed roughly the same amount of fat, over three times more than the LFD diet, while losing similar amounts of weight and liver fat. Increased consumption of fats in combination with carbohydrate restriction onset ketosis, an alternative metabolic state known for enhanced fatty acid oxidation, lipolysis, and ketogenesis (Mardinoglu et al., 2018; Donnelly et al., 2005; Luukkonen et al., 2020). In a hypocaloric context, the use of ketones for fuel would increase further as there is less caloric intake. Both ketogenic groups in this study displayed the hallmark signs of ketosis of having a reduced RER, indicating increased fat use as fuel, and markedly increased capillary ketones greater than 0.5 mmol/L. Meanwhile, the LFD consisted of a majority of carbohydrate requiring a different metabolic response. Limited dietary fat intake combined with imposed energy deficits result in less dietary fat to oxidize, a reduced need for DNL, and an emphasis on adipose tissue lipolysis for fuel, all resulting in reduced storage of fat within the liver.

The analysis of regional liver fat, measured by each segment, has been understudied in intervention literature. Due to Couinauds segmental classifications based on the idea that each segment is functionally independent with its own vascular flow (Mitra, Vikramjit, and Metcalf 2009), as well as liver fat distribution sometimes being found as unequally distributed across segments (Dulai et al., 2016) there is an obvious question if regional fat storage plays a role in post-intervention weight loss. To accomplish this, in addition to our comprehensive liver fat % we obtained for each subject, we also obtained segmental fat %. Despite other studies seeing segmental liver fat variation between regions, our study did not see such differences (Bonekamp, Tang, & Mashhood et al., 2014; Simon et al., 2015; Regnell et al., 2015). This trend continued post-intervention as each segment lost similar amounts of liver fat which also did not differ between groups. This study provided no evidence of varying levels of liver fat storage between segments nor differential responses to hypocaloric diets, or their macronutrient composition.

Similar to what others have reported (Haufe et al., 2011), select enzymes associated with liver health and function, also improved across the groups following the 6-week hypocaloric dietary intervention independent of diet composition. ALT and AST, the main liver enzymatic markers used for clinical NAFLD diagnostic referral, were lower after the diet interventions but did not reach statistical significance. Although AST and ALT are used as biomarkers of NAFLD, they have a noted lack of specificity and sensitivity of detection for NAFLD, and are used for convenience over invasive liver biopsy and still clinically uncommon advanced imaging techniques (Browning et al., 2004; Angulo, 2007). Albumin increased in the ketogenic groups and stayed the same in the LF group. Low levels of albumin are associated with and potentially predictive of liver fibrosis and NASH (Fierbinteanu-Braticevici et al., 2011, Angulo et al., 2007).

Alkaline phosphatase, another marker of liver health sometimes elevated in NAFLD patients, was seen to decrease significantly during the study in each group (McCullough, 2004; Pantsari & Harrison, 2006). ALT changes significantly correlated with liver fat changes in KD+PL and LF, while alkaline phosphatase significantly correlated with LFD, suggesting that liver fat reductions in these groups reflect liver functional changes. KD+KS did not significantly correlate with any of the liver functional enzymes supporting the theory that exogenous ketones reduce need for fatty acid delivery to the liver.

The addition of the ketone salts to the KD did not alter weight loss or liver fat responses. However, liver enzymes trended in different directions from KD + PL. Ketones have been theorized as potentially therapeutic independently from weight loss due to their signaling effects (Watanabe et al., 2020). What is of interest is if the liver fat reductions seen in this study from the KS group would occur in an isocaloric context, or if they were chronically consumed on a diet other than the KD. However, this may come at the cost of functional liver enzyme improvements. Further work into the metabolic effects of chronically consumed exogenous ketones is needed.

Twelve of the 36 participants were classified as having NAFLD at baseline using the standard criteria of having >5% liver fat. Although significant overlap exists between the population of overweight people and NAFLD, a third of our population of overweight/obese people classified as having NAFLD is surprising and concerning. None of the participants with NAFLD had prior knowledge of having liver disease. Due to the progressive nature of the disease, which can lead to death, early diagnosis is critical (Ali & Cusi, 2009). This is a significant challenge for an asymptomatic disease. Unfortunately, the gold standard of diagnosis

remains invasive liver biopsy, an invasive measure prone to sampling error that is overall suboptimal for clinical early diagnosis (Hines, Frydrychowicz, & Hamilton et al., 2011). As such, referral for diagnostic testing is usually based on elevated AST and ALT levels. However, there is considerable disagreement regarding unhealthy ranges of AST and ALT, and whether these should be used as proxy measures due to lack of sensitivity in detection of liver disease (Browning et al., 2004; Mohamadnejad et al., 2003; Kang et al., 2011). In our NAFLD subgroup, only 7 of 13 participants with had elevated ALT levels (Men > 40 U/L, women >30 U/L) (National Center for Health Statistics, 1994; Clark, Brancati, & Diehl, 2003). The development of a consensus set of clinical markers used for diagnostic referral is necessary as many individuals with NAFLD are asymptomatic and would not receive referral based on enzyme blood markers alone (Browning et al., 2004).

There is a lack of robust data pertaining to what represents a clinically significant change in liver fat. Over half of the participants who classified as NAFLD no longer met the 5% liver fat fraction threshold following the dietary intervention, likely representing a clinically relevant response. The relative decrease in liver fat in NAFLD patients was 5.7%, which also represented more than half of baseline liver fat, and is likely of clinical benefit as histological improvement have been associated with a 4% decrease in liver fat (Patel et al., 2016). A few individuals demonstrated striking reductions in liver fat. For example, one subject decreased from 10.6% to 3.4% and another from 23% to 6.3% in the KD+PL group. Notably, these striking decreases in liver fat did not translate into decreased liver enzymes. In addition to a lack of specificity and sensitivity to predicting NAFLD presence, there is a lack of reactivity to improved NAFLD condition.

A limitation of this study is that liver fibrosis was not investigated. Fibrosis can be measured using MR elastography, but we did not expect any of our subjects to have advanced stage NAFLD that would have progressed to the point of fibrosis (Taouli et al., 2007). Future dietary interventions in obese populations analyzing liver fat storage should include fibrosis measurements with the assumption there will be a subgroup classifying as NAFLD. A final limitation are the small sample sizes, due to this study being a portion of a larger overarching trial, resulting in statistics analyzing group differences having insufficient power. This is compounded within the NAFLD subgroup, which was not recruited prospectively. Having a small number of those classified as NAFLD limited the possible differences in discernable diet effects due to the majority of the population having low baseline liver fat. Finally, these diet interventions lasted just six weeks. Due to importance of weight loss for liver health improvements, it is reasonable to suggest that regaining weight would lead to liver fat reversal toward baseline. Longer term diet intervention or a study involving longer term post-diet follow-up would help to define distinctions between the diets, such as adherence and weight loss maintenance, which would have translational research value.

Conclusion

Hypocaloric ketogenic and low-fat diets prescribed for 6 weeks result in a statistically significant loss of liver fat and improvement in liver enzymes in overweight populations, and was clinically significant for over half of a NAFLD subgroup. Addition of an exogenous ketone to a hypocaloric KD did not impact liver responses. These results indicate that improvement in

liver health in the short-term is more dependent on caloric restriction than macronutrient distribution or level of ketosis. Practically, this means a variety of diets could be used to decrease liver fat in the context of moderate weight loss (5-10%).

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