THE EFFECT OF MODIFIED ALTERNATE DAY CALORIE RESTRICTION (MACR) ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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LIST OF ABBREVIATIONS

NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
HCC	Hepatocellular carcinoma
FFA	Free fatty acid
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
MRS	Magnetic resonance spectroscopy
LFT	Liver function test
APRI	Aspartate aminotransferase (AST)-to-platelet ratio
SWE	Shear wave elastography
CER	Continuous energy restriction
IF	Intermittent fasting
TE	Transient elastography
USM	Universiti Sains Malaysia
SPSS	Statistical package for the social science

BMI	Body mass index
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
TC	Total cholesterol
FBS	Fasting blood sugar

ABSTRAK

***Tajuk*:**

Kesan modifikasi kawalan kalori selang sehari pada penyakit hati berlemak bukan di sebabkan alcohol.

Latar belakang:

Penyakit hati berlemak bukan disebabkan alkohol adalah punca kepada mortaliti dan morbiditi disebabkan oleh komplikasi kardiovaskular dan juga komplikasi berkaitan dengan hati. Pada masa ini, tiada rawatan yang berkesan untuk rawatan penyakit ini; pengurangan berat badan disyorkan. Modifikasi kawalan kalori selang sehari lebih mudah dilaksanakan berbanding kawalan kalori setiap hari, akan tetapi, tiada kajian khusus yang telah dijalankan untuk melihat kesan pada penyakit ini khasnya kepada perubahan pada fibrosis dan lemak di hati.

Objektif:

Tujuan kajian ini adalah untuk menentukan kesan modifikasi kawalan kalori selang sehari pada perubahan lemak dan fibrosis di hati, anthropometri dan juga perubahan biokimia dalam penyakit hati berlemak bukan disebabkan alkohol selepas 8 minggu modifikasi kawalan kalori selang sehari .

Metod:

Kami menjalankan satu kajian prospektif yang melibatkan pesakit-pesakit gastroenterologi yang menghidap penyakit hati berlemak bukan disebabkan alkohol. Kajian ini dijalankan di Hospital Universiti Sains Malaysia dari Ogos 2015 sehingga Julai 2016.

Keputusan:

Seramai 105 pesakit telah disaring, 41 dipilih tetapi 11 menarik diri dan 30 peserta (minimun umur 43.9 tahun, BMI 31.5kg / m2, lelaki 70%, kencing manis 53%) selesai kajian. Dengan 8 minggu MACR, pengurangan ketara telah diperhatikan keatas gred steatosis hati (pengurangan 40% dalam mereka yang mempunyai steatosis sederhana, P = 0.001), SWE (perbezaan purata 0.9, P = 0.001), BMI (perbezaan purata 0.6 kg / m2 , P = 0.003), glukosa (perbezaan median 0.3 mmol / L, P = 0.01), ALT (perbezaan median 20.5 U / L, P = 0.001) dan AST (perbezaan median 9 U / L, P = 0.002)

Kesimpulan:

Kesimpulan, keputusan menunjukkan modifikasi kawalan kalori selang sehari boleh dilaksanakan sebagai strategi yang berkesan untuk penyakit hati berlemak bukan di sebabkan alkohol terutamanya ke atas pesakit dengan steatosis sederhana dan fibrosis hati peringkat awal.

ABSTRACT

Title:

The effect of modified alternate day calorie restriction (MACR) on non-alcoholic fatty liver disease (NAFLD)

Background:

Non-alcoholic fatty liver diseases are an important cause of morbidity and mortality due to adverse cardiovascular outcomes and also liver-related complications. Currently, there is no approved therapy for non-alcoholic fatty liver diseases; weight reduction is typically recommended. Modified alternate day calorie restriction is more feasible to the patient compared to daily calorie restriction; however, no trials have been done in non-alcoholic fatty liver diseases patients focusing on changes in liver fibrosis and steatosis.

Objectives:

The aim of this study is to evaluate the effect of modified alternate day calorie restriction on the changes in liver steatosis and fibrosis, anthropometry as well as biochemical parameters in non-alcoholic fatty liver diseases patients after 8 weeks of modified alternate day calorie restriction.

Methods:

We performed a prospective study involving adult gastroenterology patients with non-alcoholic fatty liver disease from August 2015 to July 2016 in Hospital Universiti Sains Malaysia.

Results:

A total of 105 patients were screened, 41 consented but 11 withdrew and 30 participants (mean age 43.9 years, BMI 31.5kg/m^2, males 70%, diabetes 53%) completed the study. With 8 week MACR, significant reductions were observed of grading of liver steatosis (40% reduction in those with moderate steatosis, P=0.001), SWE (mean difference 0.9, P=0.001), BMI (mean difference 0.6 kg/m^2, P=0.003), glucose (median difference 0.3 mmol/L, P=0.01), ALT (median difference 20.5 U/L, P=0.001) and AST (median difference 9 U/L, P=0.002)

Conclusion:

In summary, the results indicate that modified alternate day calorie restriction appear to be an effective diet strategy to help reducing the risk of progression for non-alcoholic fatty liver diseases especially in patients with moderate steatosis and mild fibrosis.

CHAPTER 1: INTRODUCTION

1.1 Non-alcoholic fatty liver disease (NAFLD) background

Non-alcoholic fatty liver disease is a chronic liver disease [Angulo 2002] with a spectrum of disease from mild hepatic steatosis to liver necroinflammation and hepatocellular injury that lead to cirrhosis later, causing liver failure and hepatocellular carcinoma as well as cardiovascular complications [Alberti 2009]. Progression of non-alcoholic fatty liver disease is highly variable, only 2-3% will progress to end-stage liver disease. However, with the rising prevalence of insulin resistance/type 2 diabetes mellitus and obesity, non-alcoholic fatty liver disease become one of most frequent liver diseases [Almeda 2009]. The spectrum of non-alcoholic fatty liver disease is shown in Figure 1.0.

stimation

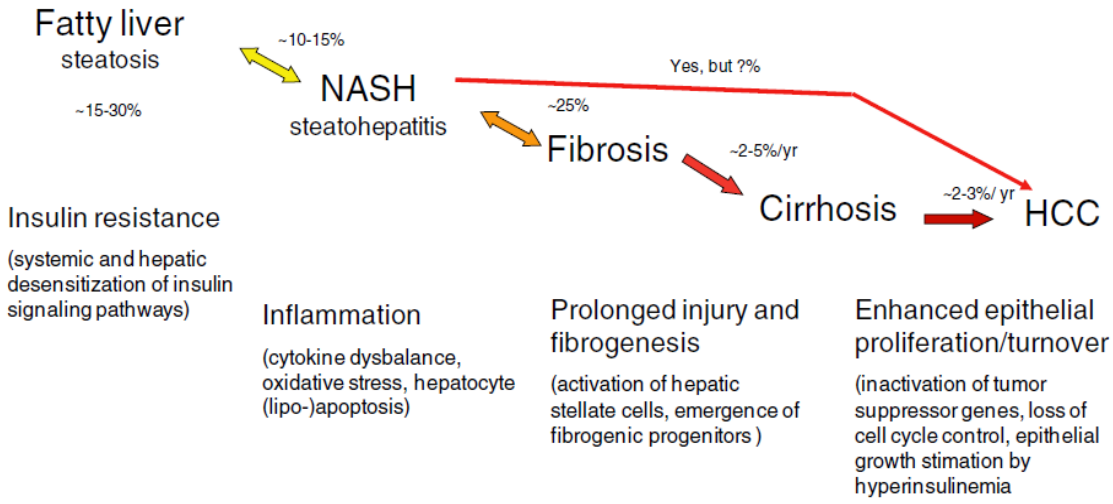

Figure 1.0 Spectrum of non-alcoholic fatty liver disease (Adapted from D Schuppan and JM Schattenberg 2013)

To diagnose non-alcoholic fatty liver disease is based on the presence of hepatic steatosis either via imaging or histology and no secondary cause for fat

accumulation such as alcohol consumption, use of steatogenic medication or hereditary disorder. Metabolic factor such as type II diabetes mellitus, obesity and dyslipidemia are commonly associated with non-alcoholic fatty liver disease [Alberti 2009].

Histologically, non-alcoholic fatty liver disease can be further categorized into two groups; non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). The presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of hepatocytes is defined as non-alcoholic fatty liver and if there is inflammation with ballooning of hepatocyte injury with or without fibrosis is defined as non-alcoholic steatohepatitis [Vernon 2011].

Non-alcoholic fatty liver disease is considered to be a benign condition with the absence of significant fibrosis; however with the presence of fibrosis [Ekstedt 2006] it does predict both disease progression and liver-related complications over a subsequent 10-year period [D Schuppan 2013]. Cardiovascular causes are predominantly leading to an increased mortality rate with a significant increase in liver-related deaths. Increase incidence of hepatocellular carcinoma in type 2 diabetes mellitus is due to high prevalence of NASH where NASH carries an increased risk for hepatocellular carcinoma [Ekstedt 2006].

Table 1.0 Prevalence of T2DM, obesity, and hypertriglyceridemia in NAFLD (Adapted from McCullough 2002)

Author	No. of patients	Diabetes (%)	Obesity (%)	Hypertriglyceridaemia (%)
Ludwig (1980)	20	25	90	67
Diehl (1988)	39	88	71	–
Lee (1989)	49	51	69	4
Powell (1990)	42	36	93	81
Bacon (1990)	33	21	39	21
Matteoni (1999)	132	33	70	92
Angulo (1999)	144	28	60	27

1.2 Pathogenesis of Nonalcoholic fatty liver disease

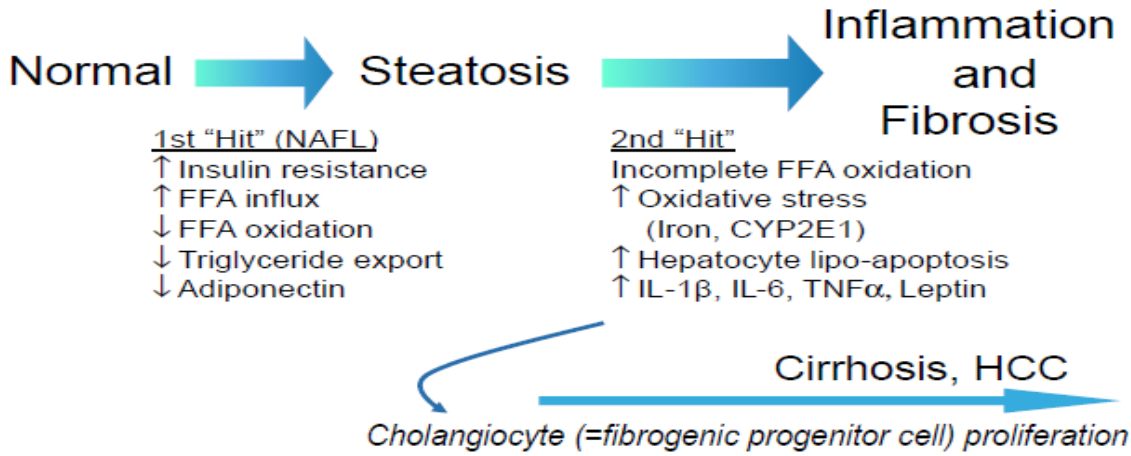

Figure 1.1 Pathomechanisms during the progression of NAFLD (Adapted from D Schuppan 2013)

Pathogenesis of non-alcoholic fatty liver disease is initially based on the '2 hit hypothesis'. Hepatic triglyceride accumulation/steatosis is considered as 'the first hit' leading to the susceptibility of the liver to injury mediated by 'second hit' such as oxidative stress, inflammatory cytokines/adipokines and mitochondrial dysfunction [D Schuppan 2013]. 'The second hit' leads to steatohepatitis and/or fibrosis. Due to the recognitions the role of free fatty acid (FFA) that plays a role in promoting liver injury, there has been a modification to this theory. In obese and insulin resistance

patients, there were influxes of free fatty acid to the liver which either undergoes b-oxidation or are esterified with glycerol to form triglycerides, causing accumulation of hepatic fat [Feldstein AE 2004]. FFA can directly cause toxicity by activation of inflammatory pathways leading to increasing oxidative stress. However, hepatic triglycerides can be a protective mechanism via preventing the toxic effect of unesterified FFA [Yamaguchi 2007]. A 'third hit' has been added to reflect inadequate hepatocyte proliferation via oxidative stress that inhibits the replication of mature hepatocytes, leading to expansion of hepatic progenitor cell (oval) populations [Joi J 2007]. Later, these cells differentiate into hepatocytes like-cell, and both oval cell and intermediate hepatocytes-like cell number that predominantly correlate with fibrosis stage. These suggest that the cumulative hepatocyte-like cell will promote both accumulation of progenitor cell and differentiation toward hepatocytes [Roskams T 2003]. Hepatocellular carcinogenesis will occur with activations of these cells. The development of fibrosis/cirrhosis depends on the efficacy of regeneration of hepatocyte with the impaired proliferation of progenitors will represent 'the third hit' in NAFLD pathogenesis [Joi J 2007].

1.3 Prevalence and natural history of non-alcoholic fatty liver disease

Differences in methods used to diagnose the various stages of non-alcoholic fatty liver disease leads to the variable estimation of prevalence. Histology features of NAFLD may be indistinguishable from alcoholic liver disease thus requiring exclusion of known excessive alcoholic intake.

International data

Screening studies in western countries using liver chemistry and ultrasonography suggest the prevalence of non-alcoholic fatty liver disease were ranging from 17% to 33% in the general population [Clark JM 2003]. Prevalence of non-alcholic fatty liver disease is increased to 34% when using magnetic resonance spectroscopy (MRS) [Browning JD 2004]. Prevalence of non-alcoholic steatohepatitis is less well known due to liver biopsy is required to confirm the diagnosis, with an estimated prevalence of approximately 3% in the general population; higher in obese persons [Wanless IR 1990].

Natural history

There are limited data looking at the natural history and progression of non-alcoholic fatty liver disease from simple steatosis to advanced fibrosis mainly due to limited number patients undergoing liver biopsies in follow-up studies. Investigation and methods to diagnose non-alcoholic fatty liver disease such as blood tests, ultrasound and liver biopsy (gold standard) [Clark JM 2003] are not uniform and long-term complication of NAFLD may be under-reported, as steatosis may disappear in the late stage of disease leading to a picture of 'bland' cirrhosis, commonly known as 'cryptogenic' rather than NAFLD-related disease. NAFLD have been recognized as the most common cause of cryptogenic cirrhosis [Clark JM 2003].

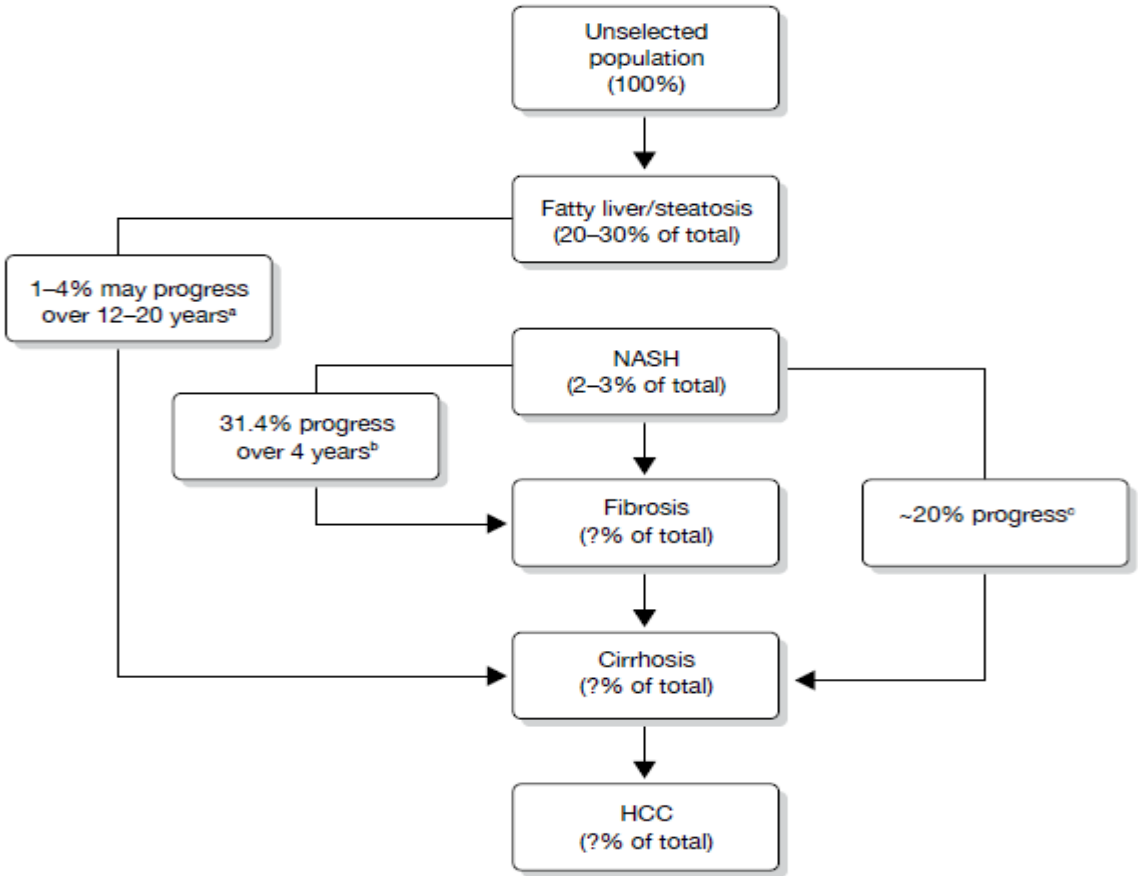

Figure 1.2 The natural history of NAFLD. (Adapted from Preiss and Satta 2008, McCullough 2002]

As a result, the majority of studies to date that have studied the natural history of non-alcoholic fatty liver disease are mainly retrospective analyses or case series in which selected patient with NASH underwent subsequent liver biopsy. It is difficult to identify the proportion of an unselected population who will progress to NAFLD-related cirrhosis and later hepatocellular carcinoma (HCC). Approximately 7% of cases of hepatocellular carcinoma were related to underlying NAFLD/cryptogenic cirrhosis [Clark JM 2003].

Associations with type 2 diabetes mellitus and obesity

The presence of type 2 diabetes mellitus leads to an increased risk and severity of non-alcoholic fatty liver disease [Targher 2007] and numerous trials have reported an increased prevalence of non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus [William RM 2009]. Up to 73.9% of subjects were found to have ultrasound-detected steatosis in 939 randomly selected people with type 2 diabetes mellitus in Edinburgh and in obese patients; the prevalence of non-alcoholic fatty liver disease is high up to 80% [Clark JM 2003]. The incidence and prevalence of type 2 diabetes mellitus and obesity is increasing in the world population leading to an increasing incidence and prevalence of fatty liver and hence non-alcoholic steatohepatitis is more likely to increase [Gonzalez ELM 2009].

Cardiovascular risk

NAFLD has been linked with increased cardiovascular complications, likely through the component of the metabolic syndrome which leads to an increased incidence of morbidity and mortality. Ghouri et al reported the presence of non-alcoholic fatty liver disease, where it is an indication for screening for type 2 diabetes mellitus, however there was no added data on cardiovascular risk as compared with traditional risk factors. Thus, it showed that non-alcoholic fatty liver disease was not an independent contributor to cardiovascular risk but it was related to adverse risk factors. A review by Targer et al mentioned the questions of whether or not non-alcoholic fatty liver disease increased cardiovascular disease with independent association with traditional risk factor. These observations raised the possibility that non-alcoholic fatty liver disease; especially with the presence of necro-inflammation,

may be involved in the pathogenesis of cardiovascular disease in addition to acting as a marker of cardiovascular disease. It may occur through the pro-atherogenic mediator that is released in the systemic circulation from a steatotic and inflamed liver, or through insulin resistance and atherogenic dyslipidemia contributed by non-alcoholic fatty liver disease. The important issue highlighted by Targher et al was that in people with non-alcoholic fatty liver disease, cardiovascular disease is greater threat than liver disease.

1.4 Clinical presentation

Clinical presentation depends on the stage of presentation of liver damage as we know that non-alcoholic fatty liver disease is a spectrum of liver damage; from mild steatosis to advanced liver fibrosis that leads to liver cirrhosis and hepatocellular carcinoma. The patient is often asymptomatic and usually detected following investigations of abnormal liver function test results. Symptoms if present may include fatigue and right upper quadrant pain and clinical finding of hepatomegaly [Sanyal AJ 2002]. Symptoms will be more prominent in people with cirrhosis, patients have clinical signs including ascites, variceal bleeding, splenomegaly, bruised and jaundice.

1.5 Diagnosis of non-alcoholic fatty liver disease

Based on clinical history and ultrasound, non-alcoholic fatty liver disease can be reasonably diagnosed, however it is difficult to stage non-alcoholic fatty liver disease and as for the current consensus for NASH; it can be diagnosed only after liver biopsy (gold standard). This would be a major hindrance to conducting a trial that requires a large number of patients for NASH.

Laboratory and radiological methods used to diagnose non-alcoholic fatty liver disease are either too insensitive or not specific to grade severity and stage it. In early steatosis, the patient is often asymptomatic and only elevated liver enzymes are the only clue to the presence of the disease. Although alanine aminotransferase (ALT) levels could be used as a reliable biochemical marker to correlate hepatic steatosis, however, up to 70% of non-alcoholic fatty liver disease patients may have normal liver enzymes [Mofrad P 2003] and it is unable to distinguish between varying stages of non-alcoholic fatty liver disease. In histologically severe disease, ALT level could be normal. An imaging method using ultrasound is recommended as a first-line investigations to detect the presence of hepatic steatosis [Ratziu V 2010]. Magnetic resonance spectroscopy (MRS) has a high sensitivity in detecting and quantifying hepatic steatosis, however, both ultrasound and MRS are not able to detect inflammation and /or fibrosis in NASH.

At present, the gold standard to diagnose non-alcoholic fatty liver disease and staging the degree of inflammation and fibrosis by histological assessment and to monitor the progression of non-alcoholic fatty liver disease is still liver biopsy [Kleiner DE 2005]. However, the limitations of liver biopsy are that it is invasive. As a response to this, multiple algorithms combining clinical and specialized biomarkers with an imaging method which uses liver elastography has been developed, this allows the use of non-invasive testing to detect inflammation/fibrosis as well as staging of the disease.

1.6 Biochemical picture associated with non-alcoholic fatty liver disease

Alanine transaminase (ALT) and aspartate transaminase (AST) are part of a group of liver function tests. In a patient with cirrhosis, AST concentration is higher than ALT and in patients with non-alcoholic fatty liver disease, if AST is raised leading to a reversal of the ALT/AST ratio, it is potentially a marker of poor prognosis, suggesting of liver cell death with inflammation (necrosis) [Preiss D 2008].

Predicting non-alcoholic fatty liver disease

Factors such as age, BMI, hypertriglyceridemia and elevated liver chemistry are independent risk factors for liver fibrosis. There are also non-invasive scoring systems that combine clinical as well as blood parameters which could be used to predict the stage of non-alcoholic fatty liver disease especially for advanced fibrosis stages [Ratviu V 2000]. Liver biopsy is an invasive procedure which may be associated with a number of complications and error in sampling, expertise is also needed to interpret the sample. This had led to efforts to find a non-invasive biomarker for fibrosis in patients with non-alcoholic fatty liver disease.

Clinical scoring systems is based on simple clinical and laboratory parameters have been suggested to assess the presence of advanced fibrosis in patients with NAFLD as well another liver diseases. These include, aspartate/ alanine aminotransferase (AST/ALT) ratio [William AL 1988], aspartate aminotransferase-to-platelet ratio (APRI) [Wai CT 2003], Fibrosis-4 (FIB-4) score [Vallet-Pichard A 2007], BARD score [Harrison SA 2008] and NAFLD fibrosis score [Angulo P 2007].

All these simple scoring systems showed high negative predictive value (NPV) in the cohort of NAFLD patient with advanced fibrosis and this suggests, simple scoring systems can be used clinically as tools to exclude patients with advanced fibrosis. Liver biopsy can be avoided in 69% of NAFLD patients in the cohort and up to 93% have been classified correctly using the simplest scoring system; the AST/ALT ratio (<0.8). Same goes to FIB-4 and NAFLD fibrosis score, liver biopsy can be avoided in half of the NAFLD patient as shown in table 1.1.

Table 1.1 Proportion of patients who may potentially avoid liver biopsy using the simple non-invasive tests to exclude advanced fibrosis (Adapted from Angulo P 1999) [41]

	Cut-off	Patients avoiding liver biopsy*	False negative result
AST/ALT	<0.8	100/145 (69%)	7 (7%)
BARD score	<2	55/145 (38%)	3 (5%)
FIB-4 score	<1.30	90/145 (62%)	4 (5%)
NAFLD fibrosis score	<−1.455	75/145 (52%)	6 (8%)

*Patients with a value below the cut-off.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAFLD, non-alcoholic fatty liver disease.

The use of these non-invasive scoring systems may substantially reduce the number of patients who undergo liver biopsies and can direct liver biopsy to those who have likely advanced fibrosis. However, in contrast to the negative predictive value, the positive predictive value for each scoring system is ranging from 27%-79% is inaccurate to be used to diagnose advanced fibrosis. As a conclusion, these simple scoring systems can be reliably used to exclude advanced fibrosis in NAFLD and can reduce the number of patients with mild disease undergoing liver biopsy [S McPherson 2010].

1.7 Imaging modalities

Liver biopsy is still the gold standard for diagnosis as well as to stage NAFLD patients and monitor the progression of the disease, however, imaging methods have gained acceptance as the non-invasive alternative to liver biopsy. Cost effective and well established imaging techniques of ultrasonography for diagnosis of hepatic steatosis is beneficial especially for screening in a large population who are at risk for NAFLD [Lee SS 2010]. It has a reliable and reasonable accuracy in detecting moderate to severe hepatic steatosis, however it is limited by operator-dependent issues as well as being less accurate in detecting mild hepatic steatosis and is rather qualitative [Palmenteri B 2006]. Computed tomography in assessing hepatic steatosis for the general population is not appropriate due to its inaccuracy in detecting mild hepatic steatosis and also is a potential radiation hazard. Other imaging modalities such as magnetic resonance spectroscopy and magnetic resonance imaging are the most accurate imaging methods in measuring hepatic steatosis which can directly measure the quantity of hepatic fat [Reeder SB 2011].

Liver steatosis grading using ultrasound modalities

Imaging modalities using ultrasonography allows for accuracy and reliability to detect the presence of moderate-severe fatty liver. Benefits are in terms of safety, low cost and easy accessibility; this imaging technique is a reliable choice for screening NAFLD in clinical and population settings. Hepatic steatosis is graded from 0-3 based on visual analysis of the intensity of the echogenicity with appropriate gain setting [Dhumal 2013].

Fat infiltration in the liver is described in 4 ultra-sonographic grades.

Normal liver parenchymal (Grade 0)

With the same kidney cortex and liver parenchyma echogenicity it is evaluated as normal, no fatty liver

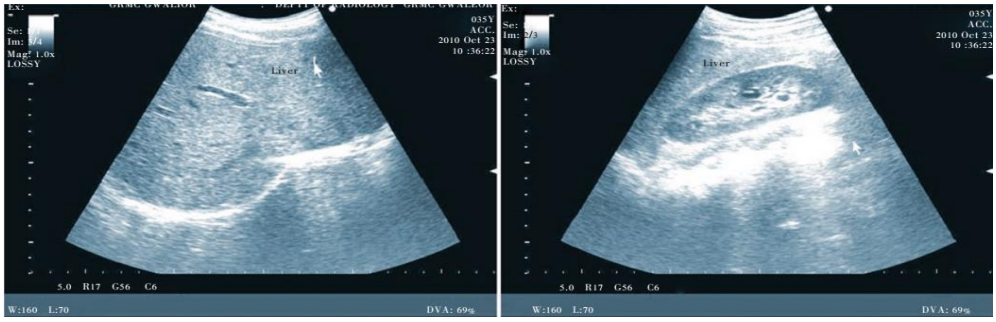

Figure 1.3 Normal liver parenchymal (Adaptation from Dhumal Uttareshvar Mahaling 2013)

Mild (Grade 1)

Increased liver echogenicity and liver-kidney contrast where liver appears bright compared to the cortex of the kidney. Normal visualization of diaphragm and intrahepatic vessel borders

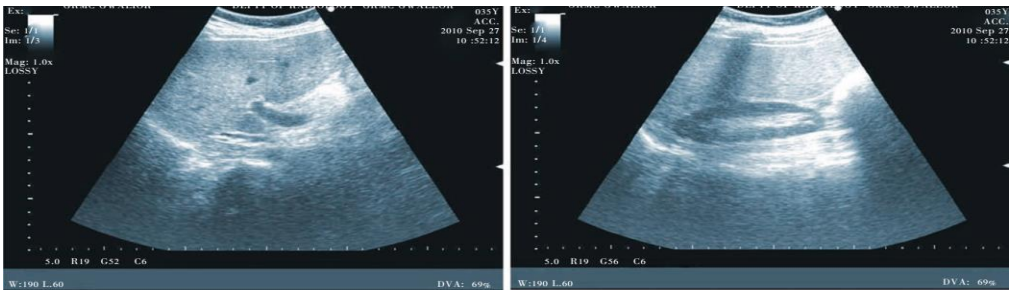

Figure 1.4 Grade 1 liver steatosis. (Adaptation from Dhumal Uttareshvar Mahaling 2013)

Medium (Grade 2)

Increased liver echogenicity and liver-kidney contrast and blurring of peripheral portal vein margins and slight deep attenuation. Slightly impaired visualization of the intrahepatic vessels and diaphragm

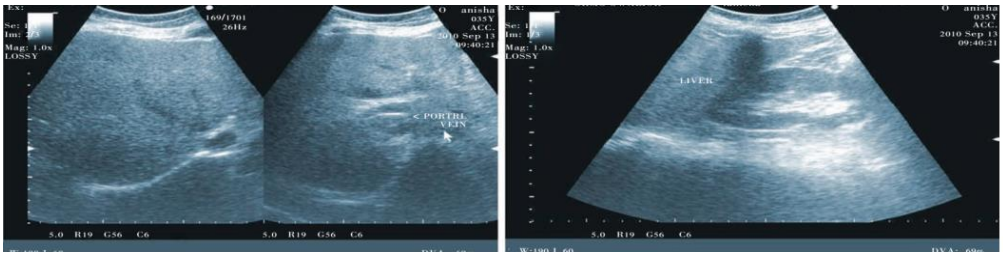

Figure 1.5 Grade 2 Liver steatosis. (Adaptation from Dhumal Uttareshvar Mahaling 2013)

Severe (Grade 3)

Increased liver echogenicity and liver-kidney contrast with non-visualisation of portal vein margins, the diaphragm and posterior part due to deep attenuation of ultrasound. Poor or no visualization of intrahepatic vessels and diaphragm with poor penetration of the posterior segment of the right lobe of the liver.

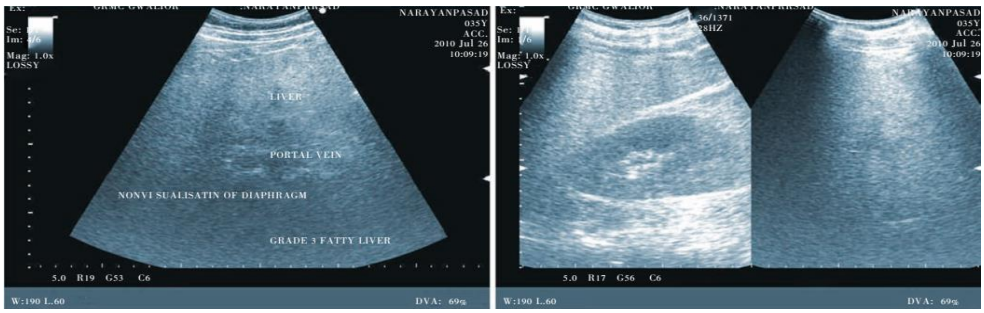

Figure 1.6 Grade 3 Liver steatosis. (Adaptation from Dhumal Uttareshvar Mahaling 2013)

The ratio between the mean brightness level of the liver and right kidney (hepatorenal sonographic index); proposed as a measure of hepatic steatosis with high sensitivity (100%) and specificity (91%) for diagnosis of non-alcoholic fatty liver disease (steatosis more than 5%) [Webb M 2009]. Furthermore, it can be integrated with liver elastography to assess liver fibrosis.

Imaging diagnosis for liver elastography

The development of steatohepatitis in non-alcoholic fatty liver disease is a risk factor for liver cirrhosis and liver-related mortality; and it is important to detect the presence of steatohepatitis/necro-inflammation before it can progress to fibrosis and cirrhosis an end spectrum of non-alcoholic fatty liver disease [Adam LA 2005]. Ultrasound and magnetic resonance elastography can be used to evaluate liver stiffness by measuring the velocity of the shear wave. Several techniques for US elastography include transient elastography, acoustic radiation force impulse elastography, real-time elastography as well as a supersonic shear wave which differ in methods of shear wave generation and /or detection. These have had been used to evaluate liver stiffness/fibrosis in patients with chronic viral hepatitis and recently its usage had been expanded to other liver diseases including non-alcoholic fatty liver disease.

Ultrasound elastography techniques have shown excellent results in evaluating the presence of liver fibrosis in non-alcoholic fatty liver disease [Fruhwirth R 2008]. As liver fibrosis increases; it will show a stepwise increase in liver stiffness and this has been highly accurate in differentiating advanced fibrosis from mild liver fibrosis.

The sensitivities and specificities of this modality are ranging from 88.9% to 100% and 75% to 100% respectively [Wong VW 2010].

The degrees of hepatic steatosis or hepatic inflammation did not correlate with a value of liver stiffness [Wong VW 2010], indicating that ultrasound elastography may evaluate liver fibrosis associated with hepatic steatosis; however, it would not be able to assess hepatic inflammation [Giovanna F 2014].

Shear wave elastography (SWE)

Shear wave elastography (SWE) is a non-invasive method to assess liver tissue stiffness. It relies on the generation of shear waves that is determined by tissue displacement which is induced by an external force or ultrasound beam. These are lateral waves with a motion perpendicular to the direction of pressure that has generated them. The shear wave travels between 1 and 10m/s and rapidly is attenuated by tissue. The elasticity of tissue correlates with propagation velocity of shear waves i.e. it increases with increasing stiffness of liver tissue.

A standard M probe is used in shear wave elastography and uses a 5-MHz ultrasound transducer mounted on the axis of a vibrator. It will generate a painless vibration with amplitude 2mm and 50 Hz frequency, propagated via skin and subcutaneous tissue into the liver and is directly related to liver tissue stiffness as shown in figure 1.8 [Giovanna F 2014].

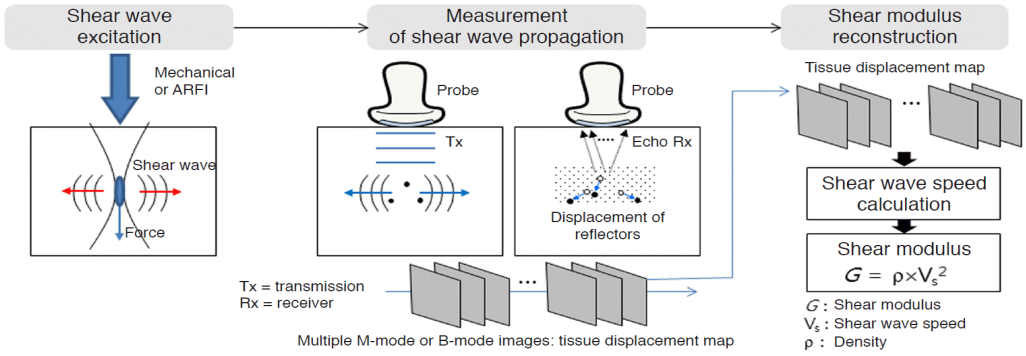

Figure 1.7 Schematic image showing physical principles of ultrasonographic shear wave elastography. (Adapted from Kema Arda 2013)

1.8 Treatment of non-alcoholic fatty liver disease

There are several effective therapies available despite understanding the pathogenesis of non-alcoholic fatty liver disease. Available treatments are focused towards improving metabolic parameters via focusing on weight loss and exercise, reducing insulin resistance by optimizing diabetic control. Others are therapies such as insulin sensitizers i.e. metformin and thiazolidinedione, weight loss drugs i.e. orlistat and sibutramine and consideration for bariatric surgery in morbidly obese patients; however none of them have been approved for general use for non-alcoholic fatty liver disease [Bugiianesi E 2002]. Even though the clinical trial of thiazolidinedione has shown positive results, however, the benefit from treatment is limited by side effects and complications of the drug, these limits widespread acceptance [Lutchman G 2007].

At the moment, there are no approved treatments, neither surgical nor medical therapy for non-alcoholic fatty liver disease. The aim of the treatment will depend on the stage of diagnosis is made as non-alcoholic fatty liver disease group. There is no

available trial that showed the outcome of treatment focusing on important long-term outcomes such development of cirrhosis or HCC.

Even with a global epidemic and a growing understanding of non-alcoholic fatty liver disease, there is still no definite pharmacotherapy available. Lifestyle modification with dietary changes and regular exercise as well as bariatric surgery in the obese patient aiming for gradual weight loss have been proposed as beneficial treatment based on available evidence [G Musso 2010]. The mainstay of treatment is still weight loss and exercise. Sustained improvement in liver chemistry, histology (inflammation/fibrosis) and serum insulin level can be achieved by weight loss and physical activities; this is recommendation for all patients with NASH, especially those who are overweight/obese [Hill JO 1999].

Pharmacological therapies that may have benefit are anti-inflammatory or anti-oxidant agents. Insulin sensitizers such as thiazolidinedione and antioxidant such as vitamin E have a promising outcome, however, there are some issues for long-term safety as well as the adverse effects that have limit the use of this drug. However it can be used in addition to lifestyle modification.

In addition, vitamin E combined with other agents may have better efficacy. Other pharmacotherapy such as pentoxifylline, ursodeoxycholic acid, and herbal medications may have potential therapeutic benefit although supporting evidence are inconclusive.

Lifestyles intervention

Evidence for weight loss focusing on improving liver histology in non-alcoholic fatty liver disease come from randomized control trials testing the effects of weight loss on nonalcoholic steatohepatitis; Pomrat et al have randomized 31 obese patients with NASH into intensive lifestyle changes over 48 weeks versus structured basic education only. The intensive group had shown significant improvement in steatosis, necrosis, and inflammation but not fibrosis. Harrison et al. showed a similar pattern where participants who had lost 5% body weight had improved steatosis, and in individuals with 9% weight loss there was significant improvement in steatosis, lobular inflammation, ballooning and NAS.

Daily calorie restriction is the commonest form of dietary restriction that is currently implemented. However even though daily calorie restriction has proven to be an effective weight loss regime especially in the obese population, people find it difficult to adhere to this strategy of weight loss [Pomprat K 2010]. Intermittent calorie restriction was invented to achieve weight loss by improving adherence with these protocols, as intermittent calorie restriction is only required every other day compared to conventional ways of daily calorie restriction [Varady KA 2007]. Intermittent calorie or also known as alternate day fasting is divided into two components, a ‘feed day’ and ‘fast day’ where food is consumed ad libitum over a 24 hours period alternating with either complete or partially restriction over 24 hours. There is also another form of intermittent calorie restriction which is 2 to 4 days of ad libitum feeding alternating with to 2-4 days of fasting or calorie restriction which could also be implemented.

1.9 Rationale of the study

The dilemma in managing non-alcoholic fatty liver disease is difficulty in staging the patient with non-alcoholic fatty liver disease due to invasive nature of liver biopsy itself, there is no approved pharmacological therapy for non-alcoholic fatty liver disease. Strong recommendations mainly focus on controlling risk factors and lifestyle intervention with physical exercise showing excellent results.

The most common diet strategies are daily calorie restriction involving restriction of energy intake every day by 20-50% [Omedei D 2011] of daily requirement per individual. However, limitation in this regime is many people find this type of dieting difficult, as vigilant calorie counting on a daily basis is required. Many people grow frustrated with this diet, as they are unable to eat freely throughout the day in the long run.

The important factor in lifestyle intervention is that sustainable and effective energy restriction strategies are required. Intermittent calorie restriction/fasting are one of the possible approaches reducing calorie requirement per day. It is a short spell of severe restriction between longer periods of habitual energy intake. This approach is much easier subject for the to comply compared to daily or continuous energy restriction (CER). However, intermittent calorie restriction itself have multiple variations in term of total calorie per day i.e. 25%, 30% and up to 40% per day, interval time of calorie restriction and also type of food i.e. polysaturated/ monosaturated fat and also variation in term of duration of calorie restriction. Furthermore, no specific trials have been done to evaluate effect of alternate day

calorie restriction in liver steatosis and fibrosis in non-alcoholic fatty liver disease patients.

By doing this study, we will able to document any significant effect of the 8 weeks modified alternate day calorie restriction which only allowed 30% of individual daily requirement of calorie per day in non-alcoholic fatty liver disease patients by evaluating the change of liver steatosis and fibrosis and also biochemical changes (lipid profile, fasting glucose, liver enzymes).

CHAPTER 2: OBJECTIVES

2.1 General Objective

The aim of this study was to evaluate the effect of 8 weeks modified alternate day calorie restriction on radiological changes in ultrasound of the liver and biochemical changes in patient with non-alcoholic fatty liver disease.

2.2 Specific objectives and hypothesis

Objective 1

Inferential Statistics

To compare differences in the median changes of liver steatosis after 8 weeks of modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.

Research question:

Is there a difference in the mean of liver steatosis before and after 8 weeks of modified alternate day calorie restriction in non-alcoholic fatty liver disease patients?

Null and alternative hypothesis:

H_0: There is no difference in mean of liver steatosis before and after the modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.

H_a: There is a difference in mean of liver steatosis before and after the modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.

Objective 2

Inferential Statistics

To compare liver elasticity using shear wave elastography (SWE) during pre and post-modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.

Research question:

Is there a difference in the mean of liver elastography value before and after 8 weeks of modified alternate day calorie restriction in non-alcoholic fatty liver disease patients?

Null and alternative hypothesis:

H_0: There is no difference in mean of liver elastography before and after the modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.

H_a: There is a difference in mean of liver elastography before and after the modified alternate day calorie restriction in non-alcoholic fatty liver disease patients

Objective 3

Inferential Statistics

To compare differences in the mean of metabolic parameters (lipid profile, BMI, blood pressure, waist circumference and fasting blood glucose) after 8 weeks of modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.

Research question:

Is there a difference in the mean of metabolic parameters (lipid profile, BMI, blood pressure, waist circumference and fasting blood glucose) after 8 weeks of modified alternate day calorie restriction in non-alcoholic fatty liver disease patients?

Null and alternative hypothesis:

H_0: There is no difference in mean of the metabolic parameters (lipid profile, BMI, blood pressure, waist circumference and fasting blood glucose) before and after the Modified Alternate Day Calorie Restriction in non-alcoholic fatty liver disease patients.

H_a: There is a difference in mean of the metabolic parameters (lipid profile, BMI, blood pressure, waist circumference and fasting blood glucose) before and after the modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.

Objective 4

Inferential Statistics

To compare differences in the mean of liver enzymes AST and ALT after 8 weeks of modified alternate day calorie restriction in non-alcoholic fatty liver disease patients

Research question:

Is there a difference in the mean of liver enzymes AST and ALT before and after 8 weeks of modified alternate day calorie restriction in non-alcoholic fatty liver disease patients?

Null and alternative hypothesis:

H_0: There is no difference in mean of liver enzymes AST and ALT before and after the modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.

H_a: There is a difference in mean of liver enzymes AST and ALT before and after the modified alternate day calorie restriction in non-alcoholic fatty liver disease patients.