Lifestyle as Therapy for Liver Disease

Jadine Hannah Scragg

Supervisory Team: Dr. Kate Hallsworth, Dr. Leah Avery, Dr. Sophie Cassidy, Prof. Mike Trenell

Doctor of Philosophy Thesis

Population Health Sciences Institute

December 2020

Abstract

Liver disease is the most rapidly growing chronic disease in the UK. 'Liver disease' comprises a vast range of aetiologies. Non-alcoholic fatty liver disease (NAFLD) is an individual aetiology that encompasses a range of disease severities, from simple fatty liver through to advanced fibrosis and cirrhosis of the liver. It is estimated that up to 33% of adults within the Western population have NAFLD, with up to 11% of those developing advanced liver disease. It is predicted that by 2030, NAFLD will be the leading indication for liver transplantation. The aim of this thesis was to assess the potential role of lifestyle intervention as a therapy for advanced liver disease including patients awaiting liver transplantation.

NAFLD is strongly associated with excessive caloric consumption, sedentary behaviour, and being overweight/obese. In the absence of approved pharmacological treatments, weight loss through lifestyle modification is the primary recommended therapy. It has been shown that a weight loss of >10% body weight is strongly associated with resolution of fibrosis, however, in practise, only a minority of patients manage to achieve and sustain this magnitude of weight loss. Following a statistical analysis whereby all thirty participants were considered, irrespective of whether they completed the intervention, Chapters 3 and 4 demonstrate that a very low calorie diet (~800kcals/day) is a safe, feasible and acceptable intervention to achieve a >10% weight loss in 34% of patients with advanced NAFLD to nine months. Chapter 5 describes differences in levels of physical activity and inactivity between patients with advanced NAFLD and age- and gender-matched healthy controls, further corroborating the need for targeted physical activity interventions for patients with NAFLD. Specifically, patients with NAFLD spent more time inactive, and less time engaging in physical activity of light, moderate, vigorous and moderate-vigorous intensities than age and gender matched healthy controls. Chapter 6 describes the development of a tailored exercise programme and an embedded retrospective cohort analysis, targeting the fitness of patients on the liver transplantation waiting list, would be acceptable to patients. The retrospective cohort analysis described clinical measures which are obtained as part of the decision making process to determine suitability for receiving a liver transplant. Data obtained from the retrospective cohort analysis demonstrated that poor cardiorespiratory fitness and other "unhealthy" lifestyle behaviours were major determinants of patients not being wait-listed for liver transplant.

In conclusion, this thesis suggests that lifestyle interventions could be employed as acceptable and feasible strategies to achieve weight loss and reduce disease progression in patients with advanced NAFLD. Furthermore, this thesis proposes that clear, structured recommendations for lifestyle behaviours be implemented into clinical care pathways for patients with lifestyle-related liver disease across the disease spectrum.

Acknowledgments

I would like to express my gratitude to a number of people without whom this body of work would not have been possible.

Firstly, Dr Kate Hallsworth, Dr Leah Avery and Dr Sophie Cassidy. Without their support, guidance, academic knowledge, and friendship, this PhD would not have happened. Specifically, Dr Kate Hallsworth, for her guidance through the world of academia as an early career researcher- thank you for helping me get my bearings in this challenging and rewarding profession. Dr Leah Avery, for her kindness and support in times of confusion and learning, and for reminding me that there are things outside of the PhD, such as our equally supportive pets. Dr Sophie Cassidy, for being there to answer all of my confused questions and for, often without intending to, providing positive feedback when I have needed it the most.

To the wonderful nurses, phlebotomy staff and secretaries at the Freeman Hospital who helped me throughout my clinical research, but most importantly at the beginning. Thank you for so kindly accommodating my confused and stressed questions and problems amongst your busy schedule. Importantly, thank you for teaching me the ways of clinical practise and always being there for me to offer a helping hand.

To each and every participant who took part in my studies, interviews and focus groups; thank you. I am enormously indebted towards you. Your enthusiasm and positivity was the biggest motivator throughout this experience and I am grateful to you all for reminding me why the past three years have been important for not just me. I hope you found the research experience to be positive and enjoyable, and that you carry the benefits with you for years to come.

To my friends, colleagues and family, most of whom will never read this, thank you for your unswerving support, even when I was thoroughly stressed and not a pleasure to be around. Thank you for being there throughout the highs and lows; you didn't know it but these friendships have dragged me through some of the most challenging days.

Guy; thank you and a thousand apologies for my rollercoaster mood swings. Words can never express my gratitude for your care and support. Thank you for cheering me on throughout the write up and helping me defeat my self-doubts.

Finally; my beloved pets. Thank you to Oreo, Cersei and Daisy for making every day filled with a little bit more love.

Publications and conference proceedings arising from this thesis

Academic Peer-Reviewed Journal Manuscripts

Scragg, J., Avery, L., Cassidy, S., Haigh, L., Taylor, G., Boyle, M., Anstee, Q, M., McPherson, S., Hallsworth, K. (2020). *Feasibility of a very low calorie diet to achieve a sustainable 10% weight loss in patients with non-alcoholic fatty liver disease.* Clinical and Translational Gastroenterology.

Taylor, G., Smith, K., Capper, T., **Scragg, J**., Bashir, A., Flatt, A., Stevenson, E., McDonald, T., Oram, R., Shaw, J., West, D. (2020). *Post-exercise glycemic control in type 1 diabetes is associated with residual β-cell function.* Diabetes Care.

Clifford, T., Hayes, E., **Scragg, J**., Taylor, G., Smith, K., Bowden Davies, K., Stevenson, E. (2020). *The effects of a high protein diet on markers of muscle damage following exercise in active older adults: a randomized, controlled trial.* International Journal of Sport Nutrition & Exercise Metabolism.

Cassidy, S., Okwose, N., **Scragg, J**., Houghton, D., Ashley, K., Trenell, M, I., Jackovljevic, D, J., Hallsworth, K., Avery, L. (2019). *Assessing the feasibility and acceptability of Changing Health for the management of prediabetes: protocol for a pilot study of a digital behavioural intervention.* Pilot and Feasibility Studies. 5, Article number: 139 (2019).

Under review

Scragg, J., Avery, L., Cassidy, S., Haigh, L., Boyle, M., Anstee, Q, M., McPherson, S., Hallsworth, K. (2020). *Patients with clinically significant non-alcoholic fatty liver disease engage in less physical activity of all intensities than matched population controls.* Submitted to Frontline Gastroenterology. Manuscript ID: flgastro-2020-101757

Peer Reviewed Published Conference Proceedings

Poster presentations

Scragg, J., Cassidy, S., Avery, L., Hudson, M., Manas, D., Prentis, J., Snowden, C., Masson, S., Hallsworth, K. (2018). *Cohort analysis of patients assessed for liver transplantation: reasons for ineligibility*. British Association for the Study of Liver Disease. September 19-21st 2018, York, United Kingdom.

Scragg, J., Avery, L., Cassidy, S., Masson, S., Haigh, L., Boyle, M., Anstee, Q, M., McPherson, S., Hallsworth, K. (2020). *Acceptability of a very low calorie diet to achieve significant weight loss in patients with advanced non-alcoholic fatty liver disease.* The Digital International Liver Conference, European Association for the Study of the Liver. August 27-29th 2020. Virtual ePoster. To be published.

Scragg, J., Avery, L., Cassidy, S., Haigh, L., Boyle, M., Anstee, Q, M., McPherson, S., Hallsworth, K. (2020). *Efficacy and feasibility of a very low calorie diet to achieve 10% weight loss in patients with advanced non-alcoholic fatty liver disease*. The Digital International Liver Conference, European Association for the Study of the Liver. August 27-29th 2020. Virtual ePoster. To be published.

Scragg, J., Avery, L., Cassidy, S., Haigh, L., Boyle, M., Anstee, Q, M., McPherson, S., Hallsworth, K. (2020). *Efficacy and feasibility of a very low calorie diet to achieve 10% weight loss in patients with advanced non-alcoholic fatty liver disease*. British Society of Gastroeneterology Annual Meeting, British Society of Gasteroloenterology. Postponed until February 8-11th 2021. To be published in Gut.

Scragg, J., Avery, L., Cassidy, S., Haigh, L., Boyle, M., Anstee, Q, M., McPherson, S., Hallsworth, K. (2020). *Feasibility of a very low calorie diet to achieve significant weight loss in patients with advanced non-alcoholic fatty liver disease*. 56th EASD (Virtual) Annual Meeting of the European Association for the Study of Diabetes. September 21-25th 2020. Virtual ePoster.

Other

Lifestyle as Therapy for Liver Disease. NIHR Newcastle BRC Trainee Showcase 2020. 18th September 2020, Newcastle upon Tyne, United Kingdom.

The relationship between your lifestyle and liver disease. Annual Soapbox Science meeting, 20th June 2020, Newcastle upon Tyne, United Kingdom. Cancelled due to COVID-19.

Table of Contents

Table of Contents

List of Figures

List of Tables

Introduction

The terminology of 'liver disease' refers to a large and pathophysiologically diverse group of diseases which primarily affect the liver. This thesis will predominantly focus on populations with non-alcoholic fatty liver disease (NAFLD) and those with end stage liver disease. NAFLD is associated with excess caloric consumption, low levels of physical activity and being overweight/ obese. Subsequently, it is estimated that up to 25% of individuals within the western population have NAFLD (Younossi et al., 2018). Likely associated with the rising levels of obesity, the prevalence of NAFLD is increasing exponentially, and is the fastest rising indication for liver transplantation (NHS, 2017, NHS, 2019). In the absence of approved pharmacological therapies for the treatment of NAFLD, the primary recommended current treatment is lifestyle modification to achieve weight loss. A weight loss of ≥10% body weight has been suggested as having the greatest likelihood of achieving resolution of fibrosis, an indicator of worsening disease status (Vilar-Gomez et al., 2015). However, in practise, only 10-20% of patients with NAFLD achieve this level of weight loss (Promrat et al., 2010).

Left untreated, NAFLD can progress from simply fatty liver to non-alcoholic steatohepatits (NASH). This is characterised by the development of worsening fibrosis, which can progress to cirrhosis and lead to cirrhotic complications. Overall, it is estimated that 5-11% of patients with NAFLD ultimately develop advanced liver disease. It is as this stage of disease progression that NAFLD is considered to an indicator for transplantation. Perioperative fitness has been shown to be a major indicator of post-transplantation survival (Prentis et al., 2012), and recent studies have indicated that exercise in this cohort of patients is safe and beneficial (Williams et al., 2019).

The overarching aim of this thesis is to explore the potential role of lifestyle behaviours as therapeutic interventions in patients with liver disease. Specifically, the data described in this thesis focuses on the potential for dietary interventions in patients with NAFLD, and the potential for an exercise programme in patients with end stage liver disease. The thesis is presented in seven subsequent Chapters. These Chapters are detailed as follows:

Chapter 1: This Chapter reviews the current literature on guidelines and therapies in patients with liver disease, with a focus on lifestyle, non-alcoholic fatty liver disease and patients with end stage liver disease awaiting assessment for liver transplantation.

Chapter 2: This Chapter details the materials and methods used to collect the data presented in the subsequent studies.

Chapter 3: This Chapter "Feasibility of a very low calorie diet to achieve a sustainable 10% weight loss in patients with non-alcoholic fatty liver disease" presents the study investigating the efficacy and feasibility of a very low calorie diet in patients with advanced non-alcoholic fatty liver disease.

Chapter 4: This Chapter presents the qualitative study investigating the feasibility of a very low calorie diet in patients with advanced non-alcoholic fatty liver disease.

Chapter 5: This Chapter presents the study investigating objectively measured physical activity and sleep data in patients with advanced non-alcoholic fatty liver disease and age/ gender matched healthy controls. It also investigated the change of physical activity and sleep following a very low calorie diet.

Chapter 6: This Chapter presents the development of an exercise intervention for patients with end stage liver disease awaiting assessment for liver transplantation.

Chapter 7: This Chapter discusses the findings presented in this thesis. Conclusions, limitations, and future studies are discussed.

Contributions from myself, research team members and other collaborators towards the data presented within this thesis

Chapter 1

I undertook the comprehensive literature review detailed in Chapter 1. However, all members of my supervisory team provided guidance towards the structure and overarching content within the review. Specifically, constructive feedback was provided by three members (Dr. Kate Hallsworth, Dr. Leah Avery and Dr. Sophie Cassidy) of the supervisory team following each iteration of the literature review.

Chapter 2

Staff from the Newcastle upon Tyne Hospitals Trust (NuTH) laboratories provided the relevant information to describe the analytical procedures used to determine biochemical data obtained from blood samples. Furthermore, NuTH laboratory staff at the Freeman Hospital undertook all analysis of the blood samples described, with the exception of the second insulin assay, as described within the Chapter. I undertook the second insulin assays using stored serum samples from the relevant study visits. Constructive feedback was provided by three members (Dr. Kate Hallsworth, Dr. Leah Avery and Dr. Sophie Cassidy) of the supervisory team following each iteration of the methods Chapter.

Chapter 3

The VLCD study was initially conceived by Dr. Hallsworth and Dr. McPherson, who acted as joint principle investigators. I conducted the majority of the ethical approval, ethical amendments, site capability and capacity evaluations, under the supervision and ratification of Dr. Hallsworth and Dr. Cassidy. Similarly, I produced the initial patient facing documents (such as patient information sheets, invitation letters, informed consent forms and FAQ documents) and initial drafts of GP letters, which were then ratified by Dr. Hallsworth to ensure complicity with NuTH and ethical committee standards. I was responsible for registering the study for CRN portfolio adoption and maintaining official records of patient recruitment and withdrawal. Pragmatic considerations with regards to study design and

data collection (for example, frequency/structure of study visits) were joint decisions between Dr. Hallsworth and myself. Alison Barnes, a dietician with Newcastle University, provided training on delivering a VLCD and advised on the use of the food replacement products. Nestle Health Sciences provided the food replacement products free of charge throughout the duration of the study. Potentially eligible patients were identified within Dietetic services or regular hepatology clinics by Prof. Anstee, Dr. McPherson, Dr. Boyle and Laura Haigh.

Within the early stages of the very low calorie diet (VLCD) study, blood samples were taken by nurses within the phlebotomy department within the outpatient services at the Freeman Hospital, NuTH. In April 2019 I became qualified and certified to undertake the required venepuncture myself within the NHS domain. However, approximately 5-10% of study visits thereafter required the support of the outpatient phlebotomy department, as I was not able to successfully draw blood at all times or from all participants. Commonly, a series of study visits were undertaken consecutively in the mornings and less commonly, in the afternoons. The majority of study visit mornings/afternoons had both myself and Dr. Hallsworth in attendance. On three occasions where Dr. Hallsworth was unavailable, Dr. Guy Taylor (a colleague within the Population Health Science Institute), attended the study visits, in order to assist with administrative tasks and anthropemtric data collection. On three occasions I managed study visit mornings/afternoons by myself, however, staff nurses at the Freeman Hospital were available should I have needed any support. Dr. Hallsworth managed one study visit morning when I was unavailable. All study administrative duties were undertaken by me, such as booking rooms for the study visits within the outpatient department of the Freeman Hospital and arranging suitable study visits for the participants. All Fibroscan measures taken at the study visits were undertaken by one of three qualified nurses at the Freeman Hospital. All other data not yet mentioned, including anthropometric data, blood pressure, body composition was generally collected between myself and Dr. Hallsworth. Prof. Quentin Anstee, Dr. McPherson and Dr. Marie Boyle all advised on safety considerations and were regularly available to consult with when concerns did arise. Decisions with regards to altering participants' medications were made by Dr. McPherson. All data analysis and visualisation was undertaken by myself, although I did consult with a

Statistician within the Faculty of Medical Sciences on two occasions to seek advice on statistical methods used within this Chapter.

Chapter 4

Within the embedded qualitative study, Dr. Avery conducted five of the semi-structured interviews at the end of the VLCD phase of the study and provided feedback on my developed topic guide. With regards to the analysis, Dr. Avery and I independently coded initial scripts to establish themes, which we then discussed with each other. At the end of the analysis, we discussed the themes once more, and the subsequent overarching thematic analysis was conducted by me and supervised and guided by Dr. Avery.

Chapter 5

Dr. Hallsworth, Dr. Cassidy and Dr. Sarah Charman, a colleague within the Population Health Sciences Institute at Newcastle University, provided advice on the study design and direction of analysis of the physical activity and sleep data. Colleagues Dr. Taylor and Dr. Charman were, at the time, conducting accelerometer-based research within healthy cohorts and we therefore worked together to age and gender match healthy volunteers to those within the VLCD NAFLD study. Initial analysis of the accelerometer data was conducted by Dr. Vincent van Hees and I conducted subsequent statistical analyses between the two aforementioned cohorts and for the data throughout the VLCD study.

Chapter 6

Data presented within Chapter 6 was in part, an element of a larger umbrella study entitled 'Lifestyle Intervention for patients undergoing Liver Transplant' (LIFT) which aimed to co-produce a multi-disciplinary lifestyle intervention for patients on the active liver transplant waiting list, with patient input. Therefore, ethical approval was obtained by Dr. James Prentis, an anaesthetist at the Freeman Hospital. Dr. Prentis identified and invited patients to attend the focus groups. Dr. Avery and Dr. Darren Flynn of Teesside University at times took the lead with parts of the focus group relating to other elements of the lifestyle intervention. I lead sections of the focus groups which related to the development of the

exercise and physical activity intervention and subsequently analysed the relevant sections of the transcript. Data used to inform the retrospective cohort analysis was available through medical eRecords. Each iteration of the exercise programme received feedback from Dr. Hallsworth and Dr. Avery, although I was responsible for developing the prototype intervention in-line with patient and health care professional input.

Chapter 7

Constructive feedback was provided by three members (Dr. Kate Hallsworth, Dr. Leah Avery and Dr. Sophie Cassidy) of the supervisory team following each iteration of all study and summary Chapters.

1. Chapter 1: Literature Review

1.1. General Introduction

Within the UK, liver disease remains the fifth biggest killer, behind cancer, stroke, CVD and respiratory disease (Murray et al., 2013), and remains the only 'big killer' that is growing in prevalence annually (Bhala et al., 2013). Liver disease can be subdivided into several different diseases including Alcoholic Liver Disease (ALD), Non-alcoholic fatty liver disease (NAFLD), Hepatocellular Carcinoma (HCC), Hepatitis, Cryptogenic Cirrhosis, Primary Biliary Cholangitis, Primary Sclerosing Cholangitis as well as many other smaller subgroups. Synonymous across the vast majority of liver diseases, fibrosis may build up as the disease progresses and ultimately lead to end stage liver disease. NAFLD is the most common worldwide, and a frequent cause of liver failure (Shaker et al., 2014). NAFLD is directly related to sedentary lifestyle, obesity and chronic excess calorie consumption. NAFLD is currently estimated to affect up to 25% of the global population, with an estimated prevalence of approximately 24% in Europe and North America (Younossi et al., 2016, Smits Mark et al., 2013). Indeed, incidence is further increased in those who are obese or who have a diagnosis of type 2 diabetes mellitus (T2DM) – i.e. it is thought that this population is three times more likely to have NAFLD. Diagnosis of NAFLD is most prevalent through the fourth to sixth decades of life. However, increases in childhood obesity has caused the rate of diagnosed paediatric NAFLD to rise. Within North America, hispanic populations are the most highly affected by NAFLD, whereas black populations have the lowest rates of NAFLD, with 48% and 18% respectively (Angulo, 2007). Approximately 40% of NAFLD patients will develop progressive liver fibrosis and ultimately up to 11% will develop end stage liver disease (Anstee et al., 2013a). Due to this increasing epidemic, it was estimated that by 2020 NAFLD would be the primary indication for liver transplantation worldwide (Bellentani, 2017). Between April 2018-March 2019, 11% of all UK liver transplant recipients had NAFLD, with alcoholic liver disease (27%) and cancer (19%) being the two most common indications for transplant (NHS, 2019).

1.2. Non-Alcoholic Fatty Liver Disease

In 1980, Ludwig et al (Ludwig et al., 1980) described a previously 'unnamed' and 'poorly understood' disease of the liver, which they proceeded to name non-alcoholic steatohepatitis (NASH). This landmark paper described a cohort of twenty patients with unknown causes of NASH, characterised by fatty changes, lobular hepatitis, mixed inflammatory infiltrates, fibrosis, and cirrhosis in three of twenty patients. It was observed that most patients were 'moderately obese' and a common comorbidity was T2DM with no known effective therapy. Today, NAFLD represents a wide spectrum of liver conditions ranging from simple steatosis (abnormal retention of lipids within the liver) to NASH and cirrhosis (end stage scarring). NASH and simple steatosis are differentiated using the presence of hepatocyte injury, inflammation and fibrosis (Dyson et al., 2014). In general, NAFLD is defined as the accumulation of fatty acids amassing greater than 5% of liver weight (Kneeman et al., 2012) in patients who do not consume excessive alcohol (Dyson et al., 2014). It is a largely asymptomatic disease, with fatigue and right upper quadrant pain being the most commonly reported symptoms (Day, 2011). It is now understood that the development of NAFLD is closely associated with lifestyle factors, such as excessive intake of hyper-caloric food and a predominately sedentary lifestyle. In recent years, the mean body mass index (BMI) in the majority of western countries has increased, as has the prevalence of obesity- which are strongly associated with the underlying pathophysiological causes of NAFLD (Marchesini et al., 2016). This is likely due to recent urbanisation, global modernisation and the consequent availability of fast food and more convenient methods of transport. Similarly, recent years have seen an adoption of 'western-style' foods in Asia and a subsequent increase of the prevalence of NAFLD (Li et al., 2019), which is further predicted to increase through to 2030 (Estes et al., 2018). Unhealthy lifestyle habits that are associated with NAFLD can be targeted to elicit significant benefits to a magnitude of lifestyle-related comorbidities. Within individuals with NAFLD, lifestyle changes to induce weight-loss can be effective at reducing liver fat, inflammation and fibrosis.

Currently, the main treatment for NAFLD is weight loss, with the goal of reducing hepatic lipid deposits and managing insulin insensitivity (Ghaemi et al., 2013), but patients often find this difficult to initiate and maintain (Avery et al., 2017). Current recommendations are vague on how best to support people with NAFLD to lose weight and often patients are left unsupported when attempting to achieve a sufficient weight loss to induce an improvement

in liver health. Recommended lifestyle behaviours, such as sustained dietary change, and increased physical activity and exercise, are not suitable for every individual and therefore it is important to establish potential barriers that individuals may face when attempting to adopt advocated changes.

1.2.1. Clinical Presentations

The vast majority of patients with NAFLD are asymptomatic; therefore, the diagnosis of NAFLD predominantly follows incidental findings of abnormal liver enzymes or imaging showing steatosis. A commonly elevated liver enzyme includes alanine aminotransferase (ALT). However, approximately 80% of patients with diagnosed NAFLD have normal-range ALT levels (Browning et al., 2004) and often elevated ALT falls as fibrosis evolves to cirrhosis. While elevated liver enzymes are associated with a clinically significant risk of developing end-stage liver disease (Ekstedt et al., 2006), elevated liver enzymes do not necessarily indicating the severity of the underlying disease (Fracanzani et al., 2008). Currently, the diagnosis of NAFLD is reliant on the exclusion of alcoholic related liver disease (ARLD), autoimmune disease, viral hepatitis or drug induced liver disease.

Following clinical suspicion, fatty deposits of the liver are commonly confirmed using ultrasonography. Ultrasound is used as a first-line investigative tool that provides a qualitative assessment of fatty acids within the liver. It is highly effective at detecting and diagnosing steatosis where over 1/3 of hepatocytes are steatotic, however, it can be unreliable with lower amounts of steatosis (Saadeh et al., 2002) and does not provide an absolute measure of the levels of liver fat. Computed Tomography and Magnetic Resonance Imaging are also accepted methods for detecting hepatic steatosis but these are not widely used in the clinical setting due to the associated expense. The major limitation of these techniques is the inability to differentiate between the histological subcategories of simple steatosis or NASH. Furthermore, they cannot identify the stage/degree of liver fibrosis (Saadeh et al., 2002). Modern approaches for diagnosis, including the use of proton magnetic resonance spectroscopy, are highly accurate at quantifying steatosis, whilst also being non-invasive. However, there is limited widespread use of this, particularly in clinical settings due to the cost (Smith and Adams, 2011).

The most definitive approach to diagnosis in NAFLD is currently liver biopsy. This provides an assessment of fibrosis, inflammation and hepatocellular injury. However, subjecting every patient with abnormal liver enzymes to this assessment is impractical and not feasible due to the cost, associated morbidity, and mortality. Therefore, the main purpose of using biopsies is to establish the stage of the disease, highlighted in Figure 1.1. Commonly, hepatology clinics limit liver biopsies to patients with some of the following (Day, 2002); ALT greater than twice normal, aspartate aminotransferase (AST) greater than ALT, T2DM, or hyperlipidaemia. Establishing the histological stage of disease is important to provide an estimate of long-term prognosis, risk of developing cirrhosis and to help direct treatment. For example, those with simple steatosis have a relatively optimistic 'liver' prognosis, with an overall 1-2% risk of developing clinical evidence of cirrhosis over 15-20 years. However, those with NASH and fibrosis can progress to cirrhosis, histologically or clinically defined, with a risk varying from 0% at five years to 12% at eight years (Day, 2006). A study using serial paired biopsies to evaluate NAFLD progression reported that over a median interval time of 6.6 years, 44% of patients with NAFLD progressed to NASH (McPherson et al., 2015). Importantly, 22% of patients with NAFLD at baseline reached stage 3 fibrosis at follow-up biopsy (McPherson et al., 2015). More recently, MRI/MRS methodologies have been employed as a means of diagnoses. McPherson *et al* evaluated the accuracy of MRI and MRS for grading the severity of steatosis (McPherson et al., 2009) and found close relationships between histology estimated steatosis and MRS/MRI. MRI/MRS also proved to be a useful diagnostic technique as hepatic inflammation and mild iron deposition did not interfere with estimation of steatosis. This could prove to be a useful tool in the diagnoses and staging of NAFLD as it is reported to be highly accurate, while having lower relative risks than liver biopsies.

The European Association for the Study of the Liver (EASL) recommend that all patients with insulin resistance and/or other metabolic risk factors, such as obesity or metabolic syndrome, should undergo diagnostic procedures, to identify fatty acid infiltrates. It is also recommended that those identified as having steatosis should be screened for secondary causes of NAFLD- namely alcohol and drug consumption. Recommendations also advise on the identification of other chronic liver diseases, as they potentially result in more severe liver injury. Similarly, National Institute for Health and Care Excellence (NICE) also identify metabolic syndrome and T2DM as risk factors and advise against using routine liver blood

tests to rule out NAFLD (NICE, 2016c). Recommendations also state that those with confirmed NAFLD should be routinely offered testing for advanced liver fibrosis every three years for adults and every two for children and young people.

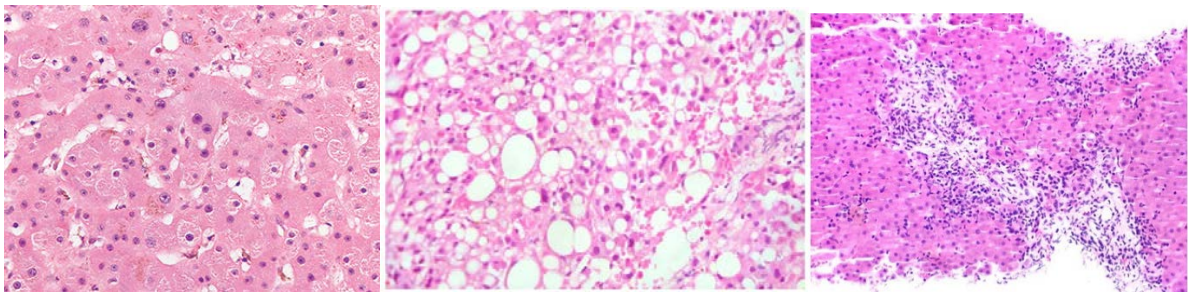


Figure 1.1: Liver biopsies showing the progression of NAFLD (from left to right) from normal liver to fatty liver and liver fibrosis

1.2.2. Pathogenesis of NAFLD

NAFLD essentially develops when the hepatic triacylglycerol (TAG) synthesis rate is greater than that of TAG catabolism. The two primary methods of hepatic TAG synthesis are; increased hepatic fatty acid uptake and subsequent esterification into TAG, and de novo synthesis of TAG from carbohydrate and protein metabolism. The methods of TAG catabolism consist of fatty acid oxidation (FAO) and TAG export as very low density lipoproteins (VLDLs). Figure 1.2 highlights the main steps in the molecular regulation of TAG turnover.

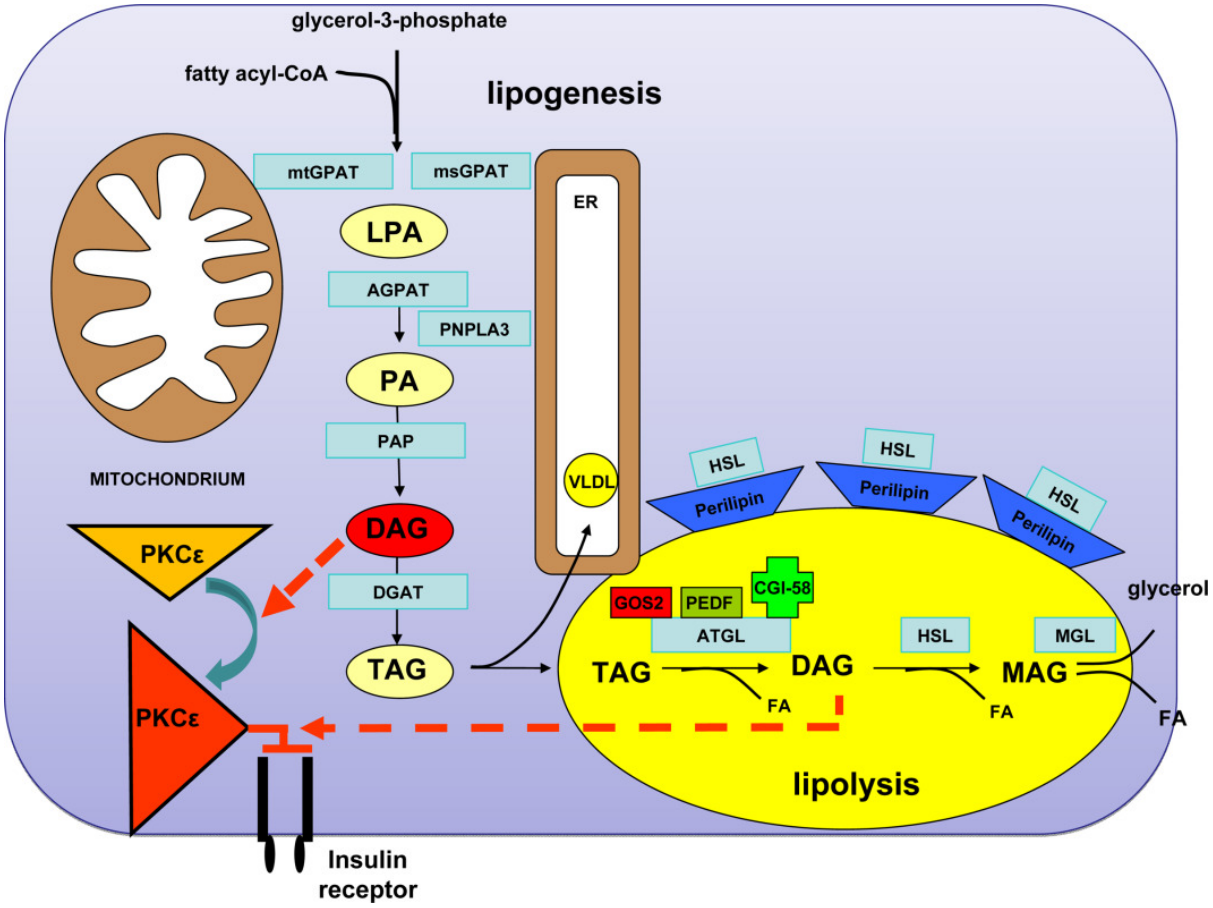


Figure 1.2: Central Pathways to TAG regulation (Birkenfeld Andreas and Shulman Gerald, 2013) *De novo lipogenesis of TAGs and phospholipids is represented by the glycerol-3-phosphate pathway. GPAT-1 (glycerol-3-phosphatase acyltransferase) catalyses the acylation with glycerol-3-phosphate to acyl-CoA, generating LPA. Phosphatidic acid phosphatase (PAP) catalyses the transfer of fatty acyl-CoAs to the glycerol backbone to form diacylglycerols (DAGs) and TAGs through diacylglycerol/ acyl-CoA acyltransferase (DGAT). DAGs in the plasma membrane fraction activate PKCε which subsequently attenuates insulin receptor activation through its ligand. TAG is hydrolysed to DAG via adipose triglyceride lipase (ATGL), and is then further hydrolysed by hormone sensitive lipase (HSL) to monoacylglycerol (MAG) and finally to glycerol by monoglyceride lipase (MGL). This sequence of reactions releases fatty acids and the resulting glycerol can be used as a substrate in gluconeogenesis (Birkenfeld Andreas and Shulman Gerald, 2013).*

It is now understood that the initial stage of NAFLD, hepatic steatosis, should be detected and targeted early to prevent complications further down the line of the progression of the disease (McPherson et al., 2009). However, the progression of NAFLD does not always follow the same linear pathway; some patients with NAFLD may never develop NASH, while conversely some show a rapid deterioration of health. Traditionally, the varying progression was thought to be due to a widely known 'two-hit' theory put forward by Day and James in 1998 (Day and James). This hypothesis states hepatic fat accumulation is the 'first hit' and the prerequisite for any hepatic injury to occur, whereas the 'second hit' can be any of bacterial endotoxin, adipokines, cytokines, mitochondrial dysfunction, or endoplasmic reticulum stress. Potential pathophysiologic mechanisms of fat accumulation within the liver include; increased fatty acid synthesis or delivery of fatty acids to the liver, reduced incorporation or removal of triglycerides as VLDL.

In more recent times, this has been challenged and revised, with the increased recognition that a combination of 'second hits' can lead to the development and progression of steatohepatitis (Levene and Goldin, 2012). This is termed the 'multiple hit hypothesis'. In this hypothesis, dietary and environmental factors, combined with obesity, lead to elevated fatty acid levels and cholesterol, insulin resistance, adipocyte dysfunction, and overall changes in the microbiome of the intestine (Buzzetti et al., 2016), all of which contribute towards increased fat accumulation and consequential lipotoxicity with the liver. The predominant fat to accumulate in those with NAFLD is triglycerides, commonly derived from esterification of free fatty acids and glycerol. The rate of de novo lipogenesis is also thought to be increased by the activation of transcription factors such as carbohydrate response element-binding protein (ChREBP) and sterol regulatory element-binding protein-1 (SREBP-1) (George and Liddle, 2008), both of which have roles within activating de novo lipogenesis (DNL) and are activated by glucose and insulin, respectively.

1.2.3. NAFLD and insulin resistance

The susceptibility of individuals to develop hepatic insulin resistance (IR) at any given level of liver fat accumulation is variable even though the biochemical mechanism is understood (Perry et al., 2014). In Figure 1.2 it can be observed that the plasma membrane diacylglycerols (DAGs) produced in the de novo lipogenesis of TAG stimulate PKCε membrane translocation which inhibits the insulin receptor kinase (Birkenfeld Andreas and Shulman Gerald, 2013).

This, in turn, leads to reduced insulin-stimulated tyrosine kinase phosphorylation of IRS-1, IRS-2 and phosphoinositide (PI3K) activation. Consequently, downstream insulin signalling is reduced resulting in an overall decline of hepatic glycogen synthesis. This is primarily due to decreased activation of glycogen synthase and increased hepatic gluconeogenesis as result of reduced inactivation of forkhead box protein O (FOXO1), thereby causing exaggerated glucose release through GLUT2, as shown in Figure 1.3.

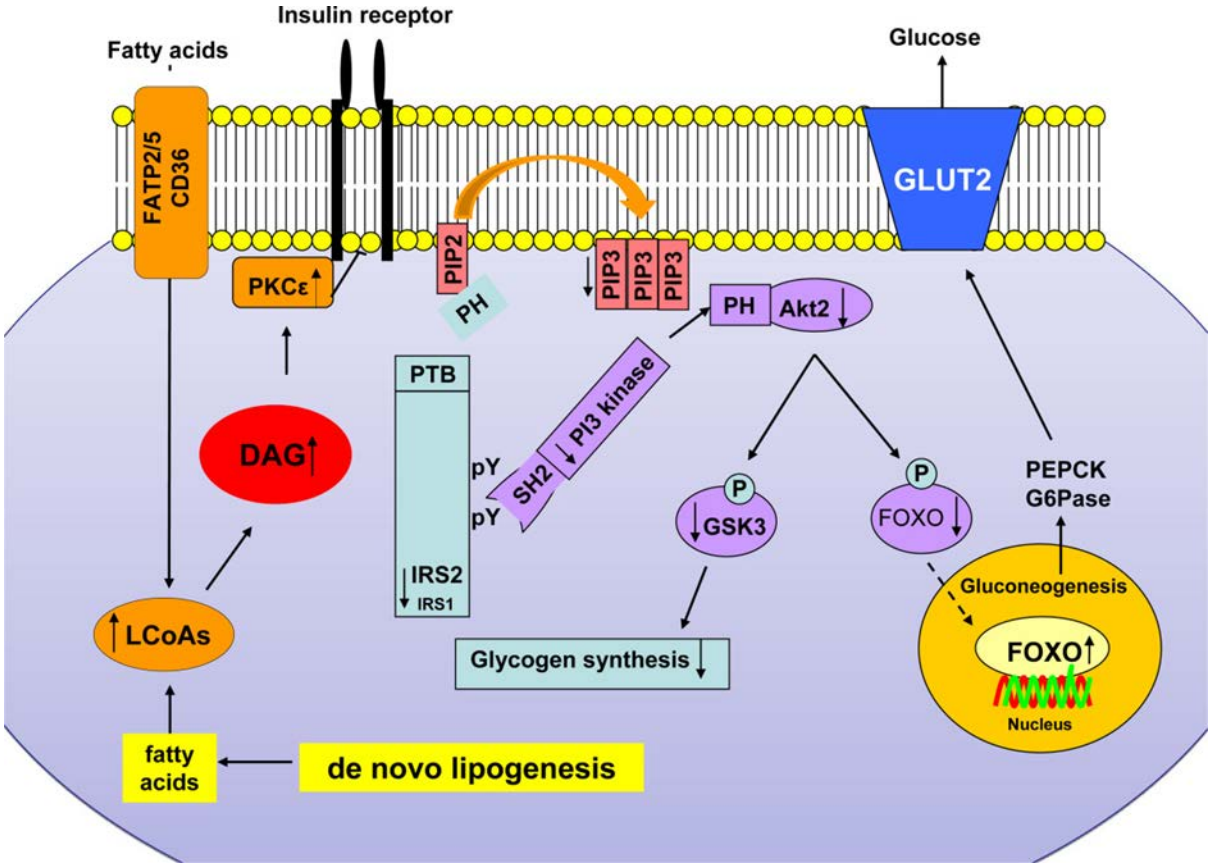


Figure 1.3: Mechanism of DAG-PKC mediated hepatic insulin resistance. (Birkenfeld Andreas and Shulman Gerald, 2013).

The plasma membrane DAGs produced in the de novo lipogenesis stimulates PKCε membrane translocation which inhibits the insulin receptor kinase, thereby reducing downstream insulin signalling (Birkenfeld Andreas and Shulman Gerald, 2013).

The variable effect is illustrated by one known genetic influence in that individuals with the G-allelle of patatin-like phospholipase 3 gene have a higher liver fat level but normal hepatic insulin sensitivity (Kantartzis et al., 2009). It is likely that other complex polygenetic traits also contribute to the variability.

1.2.4. NAFLD and Comorbidities

NAFLD and T2DM are closely related - the accumulation of liver fat can now be seen as pivotal with regards to the development of both aetiologies (Taylor, 2013), although this does vary significantly on individuals' weight and BMI (Szczepaniak et al., 2005). The prevalence of ultrasonographic NAFLD within a T2DM population was 69.4%, in an overall cohort of 180 (Leite et al., 2009), while 54% of T2DM patients were shown to have NAFLD in another study of Indian patients, diagnosed through liver biopsy and subsequent histology investigations (Prashanth et al., 2009). Further studies have investigated the correlations of the histopathological stages and T2DM, where 94% of patients presented with histologically defined NAFLD (Leite et al., 2011). The prevalence of advanced fibrosis ranged from 34% to 60% (where variance was due to ranges in fibrosis score). Independent correlates were established as older age, male gender and high serum γ-glutamyl transferase for this particular study (Leite et al., 2011), and high triglyceride, low HDL-cholesterol and increased ALT in aforementioned studies. NAFLD is essentially recognised and understood as the hepatic manifestation of the metabolic syndrome, due to insulin resistance as the underlying pathophysiological mechanism. Consequently, NAFLD is strongly associated with cardiovascular disease (CVD) as well as T2DM due to the myriad of common risk factors between CVD, NAFLD and T2DM, such as low HDL cholesterol, abdominal obesity, hypertension and chronic kidney disease (Anstee et al., 2013b).

BMI and waist circumference, measures of visceral adiposity, are positively associated with NAFLD and advancing disease (Yilmaz and Younossi, 2014). Obesity is the major phenotype for NAFLD although a small proportion of NAFLD patients are within a normal BMI range. Interestingly, many lean patients with NAFLD display insulin resistance (IR) and altered body fat distribution, while demonstrating lesser metabolic disturbances than those who are classified as obese (Vos et al., 2011). Patients with T2DM are often insulin resistant, obese, show abnormal liver enzyme portfolios, and have been shown to accumulate fatty acids within the liver independently of BMI (Gastaldelli et al., 2007). As mounting research presents the associations between NAFLD and comorbidities, recommendations regarding screening for other chronic disease have been distributed. For example, in 2012, AASLD published recommendations concerning CVD risk factors in those with NAFLD. These recommendations ascertain that clinicians must actively screen for metabolic syndrome, where an evaluation

should include fasting lipid, glucose and insulin levels as well as blood pressure and hip/waist measurements to further evaluate for hypertension and abdominal obesity. It is also recommended that patients should be questioned about CVD symptoms, namely chest pain, dyspnoea and claudication. While AASLD do not recommend routine non-invasive investigations, they conclude that consideration should be given to the Framingham risk score to identify NAFLD patients with an increased risk of CVD over 10 years (Corey Kathleen and Vuppalanchi, 2012). Due to the heightened risk of CVD within NAFLD cohorts, AASLD published recommendations on managing CVD-associated comorbidities. These recommendations are divided into lifestyle interventions and pharmaceutical approaches (Corey Kathleen and Vuppalanchi, 2012), as shown in Table 1.1. With regards to 'lifestyle interventions', the intentional loss of 10% of a patients excess body weight over 12 months is recommended via caloric reduction, reduced intake of carbohydrates and aerobic exercise. The overall summary of these recommendations maintain that NAFLD should not interfere with the treatment of other chronic comorbidities such as CVD and T2DM.

Table 1.1. Treatment recommendations for CVD risk factors in patients with NAFLD

Patient Risk Factor	**Treatment Target**	**Recommended Treatment**
CVD or risk equivalents [1]	LDL < 100 mg/dL	Lifestyle interventions and statin initiation
≥2 cardiovascular risk factors [2]	LDL < 130 mg/dL	Lifestyle interventions and statin initiation
≤1 cardiovascular risk factor	LDL < 160 mg/dL	Lifestyle interventions and statin initiation, if required
Atherogenic dyslipidemia	HDL > 40 mg/dL and triglycerides <150 mg/dL	Omega 3 fatty acids, nictonic acid and fibrates
Hypertension	<140/90 mmHg (<130/80 mmHg for diabetes or renal disease)	Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers

[1] Risk equivalents include peripheral vascular disease, carotid artery disease (CAD), diabetes mellitus and abdominal aortic aneurysms

[2] Cardiovascular risk factors include tobacco use, hypertension, a family history of premature heart disease and low HDL levels

Table adapted from AASLD 'Assessment and management of comorbidities (including cardiovascular disease) in patients with non-alcoholic fatty liver disease' (Corey Kathleen and Vuppalanchi, 2012).

1.2.5. Defining the NAFLD population

As previously discussed (see sections 1.2.1 and 1.2.2), it is likely that NAFLD is widely underdiagnosed and is estimated to affect up to 33% of the western population, with incidence likely to increase given the increasing rate of obesity. It is important to recognise the NAFLD population, as better understanding will allow for potential interventions to be targeted towards this population and therefore hopefully allow for greater efficacy. It is particularly important to define and understand the disease population as the vast majority of interventions and current recommendations for treatment pertain to lifestyle behaviours. Therefore, it is important to assess and understand the potential target population of these interventions in order to best understand the acceptability of how they may fit into the NAFLD patients' lifestyle.

There is some disparity between studies as to which gender is at a greater risk for developing NAFLD, although the general consensus is that males are at a greater risk of developing NAFLD. A range of longitudinal studies have identified males as having a greater incidence of NAFLD compared to females (Yun et al., 2016, Wong et al., 2015, Zelber-Sagi et al., 2012, Liu et al., 2013, Abenavoli et al., 2019). There is a conclusive body of evidence demonstrating that the differing prevalence of NAFLD between genders exists into older age (Lazo et al., 2013).

The primary treatment for NAFLD is lifestyle modification to reduce weight and improve fitness, with increased physical activity/exercise and a caloric deficit often recommended (see section 1.3 for more detail). However, when 'prescribing' lifestyle modification, it is important to understand any perceived barriers that a specific population may have. Understanding the socioeconomic status of a clinical cohort is of paramount importance when considering the lifestyle factors at play. There is a well elucidated association between obesity and a lower socioeconomic status (Stunkard and Sorensen, 1993, Pavela et al., 2020). The association

between the prevalence of NAFLD and socioeconomic status within the UK has not been reported, but given the strong association between NAFLD and obesity, it is likely that this association can be extended between socioeconomic status and NAFLD. Given that typical 'hard to reach' populations (Curran et al., 2016) and NAFLD populations are likely to have significant crossover, it is important to establish care pathways and interventions that are accessible and acceptable to all.

1.2.6. Limitations of Research within NAFLD Populations

A major challenge in undertaking research on NAFLD is the wide pool of NAFLD patients. It is estimated that a significant proportion of those with NAFLD are undiagnosed (Browning et al., 2004). Furthermore, there is no single consensus on the criteria for defining and monitoring NAFLD. The gold standard approach of diagnosis is liver biopsy, but biopsies are not routinely offered as part of standard care when determining the presence or severity of NAFLD. Given that NAFLD is often associated with a range of comorbidities, with obesity being the primary comorbidity, there is an inherent risk associated with performing biopsies in a large section of individuals with NAFLD (Tobkes and Nord, 1995). More simply, biopsies are expensive, invasive and are emotionally and physically demanding on individuals. Biopsy is often performed to determine the presence of cirrhosis. Therefore, where liver biopsies are unsuitable, the presence/ significance of NAFLD is often determined by a range of non-invasive scores (derived from histological evidence) such as the NAFLD fibrosis score (Angulo et al., 2007) and the Fib-4 index (Vallet-Pichard et al., 2007). However, NAFLD fibrosis score and Fib-4 score are recommended for usage when ruling fibrosis out, rather than determining presence and subsequent staging. Diagnoses may also be made using evidence derived from imaging or quantification of liver fat using proton MRI (Kramer et al., 2017). In light of this, one of the major challenges with recruiting NAFLD populations is capturing a representative example of individuals with NAFLD.

Furthermore, the challenges presented by quantifying the presence and advancement of NAFLD as outlined above are the same challenges that persist when attempting to monitor changes in liver health as a result of a lifestyle intervention.

1.2.7. NAFLD and genetics

Recently, certain polymorphisms that may be associated with NAFLD have been reported. The PNPLA3 rs738409 polymorphism has been identified as conveying an increased risk of developing NAFLD, associated with a greater reduction in hepatic triglyceride in response to lifestyle interventions (Shen et al., 2014), and increasing the risk of hepatocellular carcinoma (HCC) in patients with NAFLD, independent of the presence of cirrhosis (Liu et al., 2014b). The impaired function on TM6SF2 rs58542926 causally contributes to NAFLD; normal TM6SF2 rs58542926 activity is required for VLDL secretion and prevents an accumulation of hepatic triglyceride content (Kozlitina et al., 2014). A homozygous TM6SF2 rs58542926 minor (T) allele has been associated with a significant increase in hepatic triglyceride content (5.86% to 15.04%). Another study has associated TM6SF2 (T) allele with advanced hepatic fibrosis and cirrhosis, independent of age, BMI, T2DM and the PNPLA3 rs738409 genotype (Liu et al., 2014a). An association between TM6SF2 rs58542926 and the degree of histologically determined steatosis has also been observed (Kozlitina et al., 2014).

Similarly, carriage of GCKR rs1260326 has recently been associated with accumulation of hepatic fat, and increased levels of triglycerides and VLDLs. Furthermore, PNPLA3 rs738409 and GCKR rs1260326 have been shown to have a combined effect in conveying susceptibility to NAFLD in obese youths (Santoro et al., 2012).

However, as this is very much in its infancy, it is not yet part of routine clinical care and therefore does not affect treatment, but may explain some variations in the development of NAFLD and an individuals' responses to lifestyle interventions.

1.2.8. Pharmacological treatments for NAFLD

The role of pharmacological agents in the treatment and management of NAFLD are, as of yet, not recommended in any guidelines. Therefore managing the role of lifestyle interventions (such as diet and physical activity) are key for the management of NAFLD.

A 2010 study (Sanyal et al., 2010), evaluated the use of pioglitazone and vitamin E versus a placebo for NASH. This study randomly assigned non-T2DM subjects with NASH, to either receive 30mg daily of pioglitazone, 800IU daily of vitamin E, or a placebo. Serum ALT and AST significantly reduced in those allocated to the pioglitazone or vitamin E arms compared with

the placebo. Vitamin E and pioglitazone were associated with reductions in lobular inflammation and hepatic steatosis, however, there was no significant improvement in fibrosis scores in any group. Furthermore, those within the pioglitazone arm gained more weight than those allocated to the vitamin E or the placebo arm. The observed weight gain was a mean of 4.7 kg by week 96. The pioglitazone group experienced significantly improved insulin resistance, yet this was not maintained after discontinuation of pioglitazone, and insulin resistance returned to baseline. Weight gain was not reversed following discontinuation of pioglitazone. Given that NASH is strongly associated with obesity, it can be argued that the weight gain observed in those receiving pioglitazone diminishes the long-term effect and observed improvements. Similarly, it is likely that whichever medication is potentially prescribed for NASH, the symptoms would relapse following discontinuation of the treatment. It is probable that these medications would be prescribed indefinitely, and the observed adverse events must be carefully considered in the context of common comorbidities, namely the risk of a cardiac event. Other phase 2b trials evaluating elafibrinor and obeticholic acid (Ratziu et al., 2016, Neuschwander-Tetri et al., 2015, Friedman et al., 2016) have shown promising results in terms of improving the histological features of NASH and/or resolution of NASH without worsening of fibrosis. Reduced serum liver enzymes were observed, but these agents require more investigation to evaluate the safety and efficacy in this cohort.

Younossi *et al* recently investigated obeticholic acid as a treatment for NAFLD in the REGENERATE study (Younossi et al., 2019). Obeticholic acid is a semi-synthetic bile acid analogue and fernesoid X nuclear receptor agonist, and therefore acts to reduce the amount of bile acid within the liver. The REGENERATE study is the first positive phase 3 study in patients with NASH. Patients were randomly assigned into the placebo group, a 10mg obeticholic acid group or a 25mg dosage obeticholic acid group. Fibrosis improved in 12% of the placebo group, 18% in the 10 mg group and 23% in the 25 mg group. However, consistent with previous studies investigating obeticholic acid (Pate et al., 2019), there was a high rate of pruritus (19% in placebo group, 28% in the 10 mg group and 51% in the 25 mg group), and this caused discontinuation of the treatment in 9% of the 25 mg obeticholic acid group. Additionally, dose-dependent increases and decreases in LDL and HDL cholesterol levels, respectively, were observed; subsequently ~30% patients commenced statin therapy (66 in

the placebo group and 155 and 159 in the 10mg and 25mg dosage obeticholic acid groups, respectively). Mean reductions of ALT from baseline to 18 months were as follows: 6% in the placebo group, 26% in the 10 mg group and 33% in the 25 mg dosage obeticholic acid group. Mean reductions of AST from baseline to month 18 were as follows: 4% in the placebo group, 19% in the 10 mg group and 24% in the 25 mg dosage obeticholic acid group. Mean changes in GGT followed a similar pattern.

Other ongoing phase three studies are the REVERSE study , the AURORA study and the RESOLVE-IT study (Younossi et al., 2019, (Anstee et al., 2020). The REVERSE study is evaluating the efficacy and safety of obeticholic acid in patients with compensated cirrhosis, the AURORA study is evaluating the efficacy and safety of cenicriviroc in adults with NASH, and the RESOLVE-IT study is evaluating the efficacy and safety of elafibrinor in adults with NASH. The current primary outcome measures in all three studies is similar- the percentage of patients who achieve resolution/ improvement of fibrosis while experience no worsening of steatohepatitis. The inclusion/ exclusion criteria of pharmacological studies are strict, making for a highly selective cohort of NASH patients. For example, patients with poorly controlled T2DM are excluded from all three, with minimum HbA1c cut-offs of 9%, 9.5% and 10%. Similarly, decompensated cirrhosis is excluded in all three studies, and those with compensated cirrhosis are excluded from RESOLVE-IT and AURORA. Owing to the nature of the studies, this is likely to maximise safety, however, NASH cohorts are typically diverse in terms of comorbidities and medical history, and therefore the stringent inclusion and exclusion criteria do not allow for a true representative population of patients with steatohepatitis.

A recent study has investigated the use of prebiotics following 4 weeks of a Very Low Calorie Diet (VLCD) (600kcal/day) in patients with confirmed NAFLD (Chong et al., 2020), where follow up treatment with inulin supplementation further improved ALT scores, but did not promote further weight loss, subsequent reduction of BMI, nor any other markers of metabolic health (blood pressure and fasting blood or glucose levels).

1.2.9. Review of guidelines for the treatment of NAFLD using lifestyle interventions

National/international guidelines recommend lifestyle interventions as part of the treatment package for NAFLD, regardless of where they sit on the disease spectrum. Table 1.2 compares

the recommendations for lifestyle interventions from the NICE, European, and American guidelines (Glen et al., 2016, Chalasani et al., 2018, NICE, 2016c).

Table 1.2. Recommendations on the management and treatment of NAFLD

AASLD (Chalasani et al., 2018)	EASL (NICE, 2016c)	NICE (Glen et al., 2016)
• Pharmacological treatments aimed primarily at improving liver disease should generally be limited to those with biopsy-proven NASH and fibrosis. • A combination of a hypocaloric diet (daily reduction by 500-1,000 kcal) and moderate-intensity exercise is likely to provide the best likelihood of sustaining weight loss over time. • Weight loss of at least 3%-5% of body weight appears necessary to improve	• Modest caloric reductions of 500-800kcal/day to be accompanied by a daily protein intake of 1.2-1.5 g/kg of body weight. * • As above, combined with a regular physical activity/ exercise programme • Structured programmes aimed at lifestyle changes towards healthy diet and habitual physical activity are advisable in NAFLD • Patients without NASH or fibrosis should only receive counselling for healthy diet and physical activity and no pharmacotherapy for their liver condition • In overweight/obese NAFLD, a 7–10% weight loss is the target of most lifestyle interventions, and results in improvement of	• Explain to people with NAFLD who drink alcohol the importance of staying within the national recommended limits for alcohol consumption. • Explain to people with NAFLD that there is some evidence that exercise reduces liver fat content independently of weight reduction. • Offer advice on physical activity and diet to people with NAFLD who are overweight or obese in line with NICE's obesity

steatosis, but a greater weight loss (7%-10%) is needed to improve the majority of the histopathological features of NASH, including fibrosis. • Exercise alone in adults with NAFLD may prevent or reduce HS, but its ability to improve other aspects of liver histology remains unknown.	liver enzymes and histology • Dietary recommendations should consider energy restriction and exclusion of NAFLD-promoting components (processed food, and food and beverages high in added fructose. The macronutrient composition should be adjusted according to the Mediterranean diet • Both aerobic exercise and resistance training effectively reduce liver fat. The choice of training should be tailored based on patients' preferences to be maintained in the long-term	and preventing excess weight gain guidelines. • Do not offer omega-3 fatty acids to adults with NAFLD because there is not enough evidence to recommend their use.

*advice specific to patients with cirrhosis

Given the strong relationship between hypocaloric diets and the exacerbation of sarcopenia (Yanai, 2015a), excessive caloric restriction is not widely advocated in patients with NASH-related cirrhosis. Sarcopenia has been identified as a major predictor of poorer outcomes in obese patients with cirrhosis (Eslamparast et al., 2018). Table 1.3 summarises the recommendations when BMI, waist circumference and comorbidities are accounted for.

Table 1.3: NICE recommendations for lifestyle intervention

BMI Classification	**Waist circumference**			**Comorbidities**
	Low[1]	High[2]	Very High[3]	
Overweight	1	2	2	3
Obesity I	2	2	2	3
Obesity II	3	3	3	4
Obesity III	4	4	4	4

[1] low waist circumference is classed <94cm for men, <80cm for women

[2] high waist circumference is classed as 94-102cm for men, 80-88cm for women

[3] very high waist circumference is classed as >102 for men, >88cm for women

3. General advice on healthy weight and lifestyle
3. Diet and physical activity
3. Diet and physical activity; consider drugs
3. Diet and physical activity; consider drugs; consider surgery

1.3. The evidence for lifestyle interventions (Diet and Exercise) in NAFLD

1.3.1. Weight loss

The primary treatment for NAFLD is weight loss, with a prominent study showing that weight loss of 3-5% is associated with a reduction in hepatic steatosis, 5-7% is associated with improvement of inflammation, and over 10% weight loss can improve hepatic fibrosis (Vilar-Gomez et al., 2015). In this study of routine clinical care, weight-loss was induced by advice to follow a low-fat hypocaloric diet (750kcal/day less than the recommended daily allowance) combined with 200 min/week of low intensity physical activity, in a population of 261 participants with NASH as indicated by biopsy. This study also established the likelihood of achieving NASH resolution and fibrosis regression proportionate to weight-loss, determined using a repeat biopsy 12 months from baseline measures. The likelihood of NASH resolution was 10% and 90% in those who lost <5% and >10% of total body weight, respectively.

Similarly, the likelihood of fibrosis regression was 16% in those who lost <5%, and 45% in those who lost >10% of total body weight. An intervention evaluating a low-fat, low-Glycemic Index (GI) diet combined with up to 150 min/week of moderate intensity physical activity demonstrated reversal of NAFLD in 50% of those who lost 5.0-6.9% of bodyweight, 60% of those who lost 7.0-9.9% and in 97% of those losing 10% of more of their starting body weight (Wong et al., 2013). Interestingly, baseline BMI and intrahepatic triglyceride levels have been identified as contributing factors in the potential resolution of NAFLD. Following a 3-5% reduction in weight, 50% of non-obese (determined by BMI) participants had achieved remission of NAFLD, however, in obese participants, a weight reduction of 7-10% was required to induce NASH resolution in 50% (Wong et al., 2018).

In a histologically proven NASH cohort, weight loss has also been shown as an effective technique in reducing fat, with the greatest weight loss associated with the best improvements histologically on a repeat biopsy (Vilar-Gomez et al., 2015).

While this level of weight loss has been shown repeatedly to be efficacious in inducing reduction in liver fat and improve necroinflammation (Chalasani et al., 2012), it has been observed that many patients struggle to achieve weight loss of ≥7%. A recent community and internet-based intervention showed that only 20% of participants were able to lose >10% of their starting body weight over 1-2 years (Mazzotti et al., 2018). For example, Musso et al (Musso et al., 2012) evaluated the effect of weight loss on NAFLD patients and this meta-analysis articulated that less than half of the patients were able to achieve this weight loss target. A recent non-interventional study by Vilar-Gomez et al., (2015) assessed paired biopsies of patients, and showed only 30% of patients had lost ≥5% of their total body weight over 1 year. Of those that had lost weight, 57% had resolution of their NASH and 82% had a 2 point reduction in NAFLD activity score (NAS)- a composite score derived using steatosis, inflammation and hepatocyte ballooning, determined using liver biopsy. Furthermore, 29 patients (of 293) achieved ≥10% weight loss, and of these, 100% had a reduction in NAS, 90% had resolution of NASH and 45% achieved regression of fibrosis, as shown in Figure 1.4. In another, smaller study (Glass et al., 2015) 45 patients were assessed for fibrosis regression (defined in this study as an improvement in fibrosis score ≥1 stage using the NASH Clinical Research Network guidelines). They concluded that fibrosis regression is possible, even in those with advanced disease and that weight loss of ≥10% total body weight predicts

regression of fibrosis. Of interest, 12 of the 45 total patients underwent bariatric surgery and the rate of fibrosis regression was significantly higher in those particular patients.

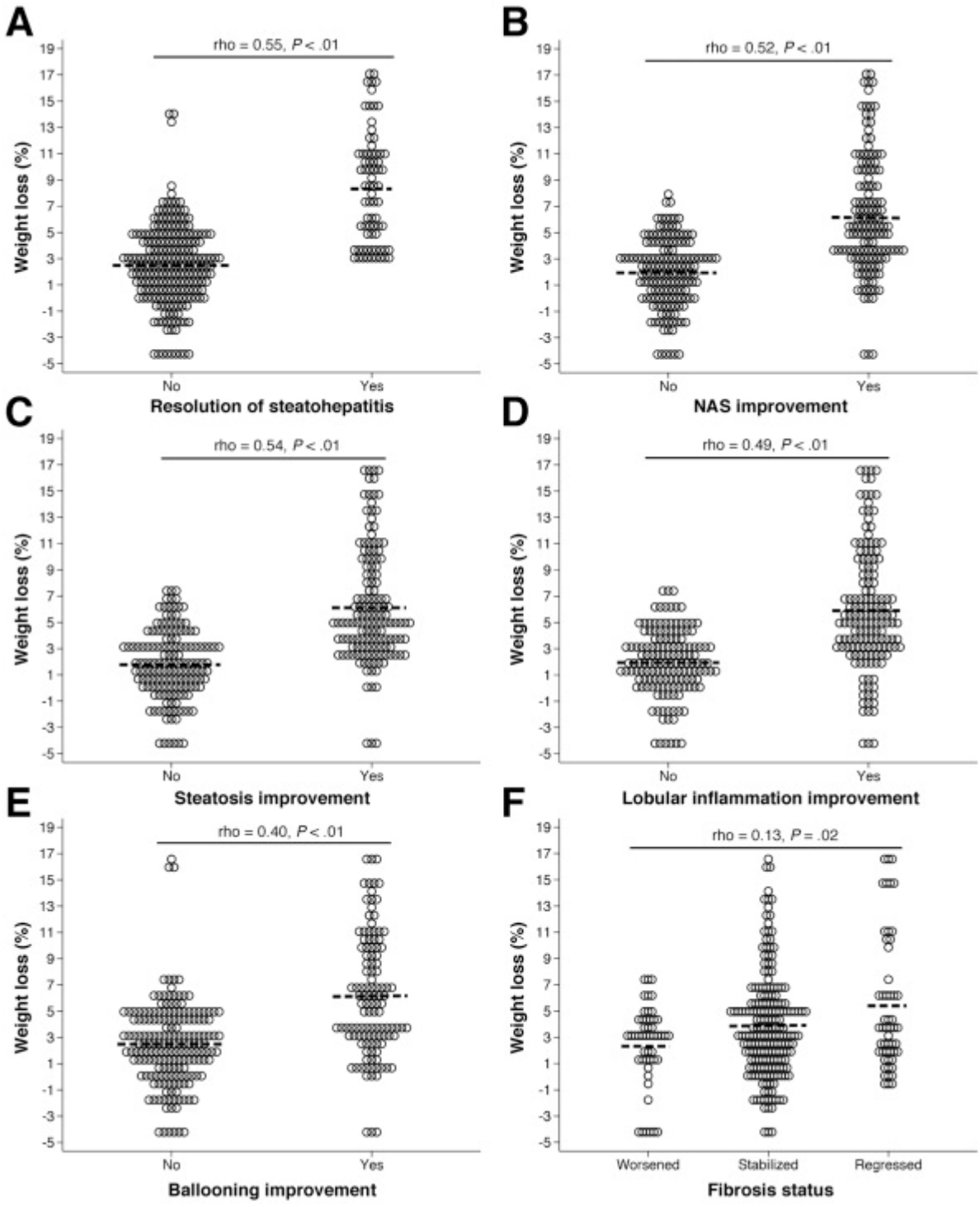


Figure 1.4. Correlation between weight-loss percentage and improvement of different histologic parameters related to NASH. (A) Resolution of steatohepatitis, (B) NAS improvement, (C) steatosis improvement, (D) lobular inflammation improvement, (E)

ballooning improvement, (F) Fibrosis status (Vilar-Gomez et al., 2015). Published with permission from Vilar-Gomez et al., 2015.

1.3.2. Diet

Within regards to dietary lifestyle interventions, the most effective weight loss strategies have not been defined. Often, dietary recommendations are in line with those recommended by for the management of Diabetes by the American Diabetes Association (ADA) (NICE, 2016a). Weight loss inducing diets are frequently subdivided into four categories; low fat, low carbohydrate, low calorie and very low calorie diet (VLCD).

Low-carbohydrate and low-fat diets have gained interest in recent years, as safe and viable options for inducing weight loss and maintaining it in both clinical and non-clinical populations. Haufe et al (Haufe et al., 2011a) investigated the effect of low carbohydrate and low fat diets on hepatic lipid content in overweight and obese patients. This trial randomised 170 patients to either diet for 6 months. Of the 102 that completed the intervention and underwent magnetic resonance spectroscopy of the liver, both low fat and low carbohydrate diets achieved similar decreases in intrahepatic lipid content and total body weight. However, these conclusions are not specific to NAFLD patients.

There is limited data of the effect of differing dietary components in NAFLD populations, however, one small pilot (Tendler et al., 2007) study of five participants investigated the effects of the low carbohydrate diet in biopsy confirmed NAFLD. Patients were instructed to follow a <20g/ day carbohydrate diet for six months. At the end of the intervention four of the five biopsies showed significant improvement in steatosis ($p = 0.02$) and inflammatory grade ($p = 0.02$) as well as a trend towards fibrosis improvement ($p = 0.07$). However, due to the small numbers of this study and lack of a comparative group, it cannot be reliably concluded that these results were due to the diet or the weight loss achieved.

The most recommended dietary pattern for patients with NAFLD to follow is a Mediterranean style diet. A Mediterranean style diet typically constitutes a high intake of olive oil, nuts, vegetables, fish, legumes and fruits (Zelber-Sagi et al., 2017). Traditionally, adherence to a Mediterranean style diet has been associated with a reduction in risk of CVD. Given that the common cause of death in patients is CVD related, it is therefore an appealing dietary regimen. A significant reduction of BMI, weight, cholesterol and triglycerides has been

observed in patients with NAFLD following a Mediterranean dietary intervention, but no changes has been shown in HDL, LDL, glucose, blood pressure or insulin (Biolato et al., 2019, Abenavoli et al., 2017, Katsagoni et al., 2018). Similarly, a Mediterranean diet has also been shown to improve insulin sensitivity and hepatic steatosis (Ryan et al., 2013). Other studies have also shown that adherence to a Mediterranean style diet can elicit a reduction in liver fat in the absence of weight loss (Romero-Gómez et al., 2017). Overall, two randomised control studies have reported that hepatic triglycerides are lowered more so by a Mediterranean style diet than a low fat diet (<30% of daily caloric intake from fat) (Gepner et al., 2019, Ryan et al., 2013).

Aside from using dietary changes to induce weight-loss, altering the components of an individual's diet has been shown to improve liver health, both with and without weight loss. For example, saturated fats, often associated with hypercaloric diets, have been shown to increase de-novo lipogenesis and subsequent hepatic fat content, when compared to unsaturated fats and carbohydrates (Luukkonen et al., 2018). It can therefore be assumed that a diet higher in saturated fats is likely to be harmful to overall liver health. As previously discussed (see 1.2.2), higher levels of circulating free fatty acids, often dietary derived, are important pathophysiological mechanisms in the development of NAFLD. Similarly, studies have demonstrated that high fat/ low carbohydrate (43-56%/30-38%) diets increase triglyceride content within the liver, compared to a low fat/ high carbohydrate diet (16-23%/57-65%) (Yki-Järvinen, 2015). High protein diets, either animal or plant-based sources (>30%) have been shown to reduce hepatic fat and hepatic steatosis over a 6 week period (Markova et al., 2017, Skytte et al., 2019). When sustained for a longer period of time (up to 6 months) hypocaloric diets lead to the same reduction of triglycerides within the liver. This has been achieved using low carbohydrate (10-30%) or low fat (20%) diets (Kirk et al., 2009, Haufe et al., 2011b).

There is debate over the suitability of VLCD as a means for weight loss induction in NAFLD cohort. Most studies have concluded that VLCD is a safe and viable approach for weight loss in NAFLD patients and can safely be administered without adverse events (Temmerman and Friedman, 2013). In a recent study at Newcastle University, The Diabetes Remission Clinical Trial (DiRECT), following 8 weeks of restricted energy intake (VLCD), fasting plasma glucose normalised, insulin suppression of hepatic glucose output improved and hepatic

triacylglycerol content fell from 12.8 ± 2.4% to 2.9 ± 0.2% in a T2DM cohort. No adverse events were documented throughout the entire 8 week intervention (Leslie et al., 2016).

1.3.3. Physical activity/exercise

Commonly, weight loss is achieved through a dietary, exercise, or dietary plus exercise intervention. Evidence supporting lifestyle interventions as therapy is strong and has conclusively shown significant reductions in hepatic fat as well as improvements in insulin sensitivity/ glucose control following dietary and exercise interventions (Thoma et al., 2012), as well as improvements in cardiovascular disease risk factors, such as serum lipids and HbA1c (St. George et al., 2009, Gepner et al., 2019, Sun et al., 2012, Wong et al., 2013, Promrat et al., 2010, Eckard et al., 2013, Ueno et al., 1997).

NAFLD cohorts were, until fairly recently, anecdotally less active than their healthy counterparts. However, in recent years there have been a plethora of publications reporting, using a variety of means and methods, on the physical activity of NAFLD populations. Physical activity has previously been reported as lower within NAFLD cohorts than within healthy cohorts, where mean step count (steps/day) were significantly lower in the NAFLD subgroup (Newton et al., 2008). However, for a more detailed description, accelerometry is frequently used over step counts, as it allows the research team to objectively measure and categorise physical activity (PA) into different categories, such as light, moderate or vigorous. It also allows for time spent partaking in sedentary behavior to be quantified, which in itself has been identified as being associated with obesity and increased cardiovascular risk (Vainshelboim et al., 2017, Ekelund et al., 2019). Objectively measured time spent in sedentary behavior has been associated with reduced energy expenditure and therefore provides a lifestyle behavior that could be targeted as a therapeutic means to reduce weight gain in individuals with NAFLD (Hallsworth et al., 2015). For example, Gerber *et al* used triaxial accelerometry to evaluate activity in NAFLD vs. non-NAFLD cohorts (Gerber et al., 2012). This was a large study with >3000 participants split between NAFLD and non-NAFLD cohorts, in which it was reported that average PA for NAFLD participants was approximately 29 counts/minute/day less than controls ($P < 0.01$), with NAFLD subjects spending less time participating in activity at any level ($P < 0.01$). Individuals with NAFLD and T2DM were in the lowest quartile of both average and moderate-vigorous PA ($P < 0.01$). However, while this was

reported in a large (>3000) population, the comparator arm was not age or gender matched to the NAFLD cohort, and each individuals PA was only captured for 10 hours/day. Arguably, 10 hours/day is a restrictive time window and could significantly over- or underestimate an individuals' physical activity. NAFLD was defined using fatty liver index (FLI), which is calculated using BMI, therefore it could be argued that those who are obese may wrongly be analysed as part of the NAFLD cohort and therefore may not be entirely representative of a NAFLD cohort.

While physical activity is commonly used to induce/maintain weight loss, it is important to establish the effect that significant weight loss has on free living physical activity in individuals with NAFLD. There are limited data evaluating this in any disease cohort, however one study reported that following surgically induced weight loss (in a typical obese cohort), physical activity significantly increased, and this increase correlated with the amount of weight lost over a two year follow-up period (Kárason and Van Der Hilst, 2000). However, this study is largely limited in that PA data was determined using self-report, an approach that has been widely reported to elicit results which are over or under-estimates of objectively monitored PA measures (Shephard, 2003). Another study objectively evaluated PA changes in obese patients pre- and post-bariatric surgery using an accelerometer, and reported no significant changes in overall PA, or in mean moderate-vigorous bouts of PA after significant weight loss at 6 months post-surgery (Afshar et al., 2017).

While interest in, and the subsequent research of, the PA of NAFLD cohorts has increased, there are still areas in which further work is needed. For example, when determining how NAFLD populations compare to the healthy counterparts, large populations are needed where age, gender and, if possible, BMI are matched between NAFLD and comparator arms. Given that a higher BMI is more likely in a NAFLD cohort compared to a healthy population, BMI matching could be challenging, particularly as it could be difficult to find 'healthy' individuals with a higher BMI. Additionally, it is important that 24 hours, or as close to, of data is captured per day, to provide a fuller picture of individuals' PA. Given the high proportion of individuals who are employed in shift-work, it is necessary to record for, as close to as possible, 24 hours/day for as many consecutive days as possible to best represent all individuals. Finally, given the aforementioned difficulty of conducting research in NAFLD cohorts (see section

1.2.6), it is necessary that those classified as having NAFLD have received a definitive diagnosis.

Both aerobic and resistance exercise have been shown to reduce liver fat and its mediators in patients with NAFLD, independent of weight loss (Hallsworth et al., 2011, Johnson et al., 2009). Given that both are effective, and that resistance exercise requires less energy, it may be that resistance exercise is more appropriate for patients with NAFLD who have poor cardiorespiratory fitness.

1.3.4. Combined diet and physical activity

Promrat *et al* (Promrat et al., 2010) evaluated the effect of a low-fat (25%) diet, combined with 200 min/week of moderate intensity PA and cognitive behavioural therapy (CBT) over a 48 week period. This intervention elicited a mean weight loss of 8.7 kg, improved steatosis and NAS, while no difference was observed in glucose or HOMA-IR. Wong *et al* (Wong et al., 2013) observed a 7% reduction in hepatic triglyceride, achieved using a low-fat, low-GI diet combined with 210 min/week of moderate intensity. Similarly, Gepner *et al* (Gepner et al., 2019) observed reductions of 6% and 7% in hepatic triglycerides in a low-fat intervention and a Mediterranean diet combined with 180 min/week physical activity, respectively. Weight loss orientated lifestyle interventions have shown reductions in liver fat of 42-81% with a strong correlation being shown between amount of weight lost and the volume of liver fat reduction (Kirk et al., 2009, Viljanen et al., 2009) .

A 16 week lifestyle intervention (Berzigotti et al., 2017) comprising of a personalised hypocaloric diet combined with 60 min/week of supervised physical activity in individuals with compensated cirrhosis and portal hypertension showed that weight loss by the end of the intervention was maintained at follow up (6 months). A >10% reduction in body weight was associated with a greater decrease in hepatic venous pressure gradient (HVPG). 52% of participants lost over 5% of their starting body weight, while 16% lost over 10% and HVPG decreased by >10% in 42% of participants and >20% in 24%. No episodes of clinical decompensation were observed throughout the intervention, thereby indicating a lifestyle intervention in obese individuals with compensated cirrhosis is not only safe, but also significantly efficacious at reducing body weight and portal vein pressure. Overall, 60 participants were recruited and 50 completed the study. Going forward, longer studies with larger patient numbers are needed to full assess the role of lifestyle interventions on various indicators of liver health.

1.3.5. Recommendations for weight loss in NAFLD

Recommendations for lifestyle interventions for the management of NAFLD generally include both diet and exercise to combine decreased caloric intake and increased energy expenditure. According to a meta-analysis by Wu et al (Wu et al., 2009), combined diet and exercise interventions resulted in a mean loss of 1.14 kg greater than the diet only interventions in longitudinal studies of over two years. However, this data should be treated with caution on

this occasion as this is not unique data to patients with NAFLD. However, with the absence of approved pharmacological therapies, lifestyle interventions remain the leading therapies for patients with NAFLD. Until larger, prospective, randomised clinical trials have compared the effects, in NAFLD cohorts of exercise alone, diet alone and exercise and diet combined interventions, formal recommendations between the three cannot be made. A major limitation is patient variability- many studies only assess general physical activity or diet, which therefore does not allow for cross comparison between studies.

1.3.6. Problems with sustaining weight loss

While the predominant recommendation for the treatment of NAFLD is to elicit significant weight loss through dietary and PA/ exercise modification, this does not suitably address the challenges that many individuals face in terms of maintaining weight loss. A plethora of studies have indicated that maintaining significant weight loss is challenging and the proportion of patients who successfully do so is often low; estimates of long term maintenance of significant weight loss vary from 20% of patients sustaining >10% weight loss (Wing and Phelan, 2005), 24% of patients sustaining >10 kg at 24 months (Lean et al., 2019) to 87% of participants maintaining >10% weight loss at 5 years (Thomas et al., 2014). The range in these varying levels of success can potentially be explained by the diversity of populations and modes of recruitment. For example, Thomas *et al* recruited participants who had previously lost 30lb (13.6 kg) and maintained this for 1 year through newspaper (or other various media) selection. Questionnaires were sent to participants annually, and therefore dependent on self-report. Given that these participants had already successfully maintained weight loss for more than 1 year, it may be that they had surpassed the 'difficult' period of weight maintenance. Figure 1.5 depicts the weight loss and subsequent weight loss, achieved using various methodologies of >26,000 participants, where it can be seen that historically patients who have undertook a VLED experience a greater proportion of weight regain.

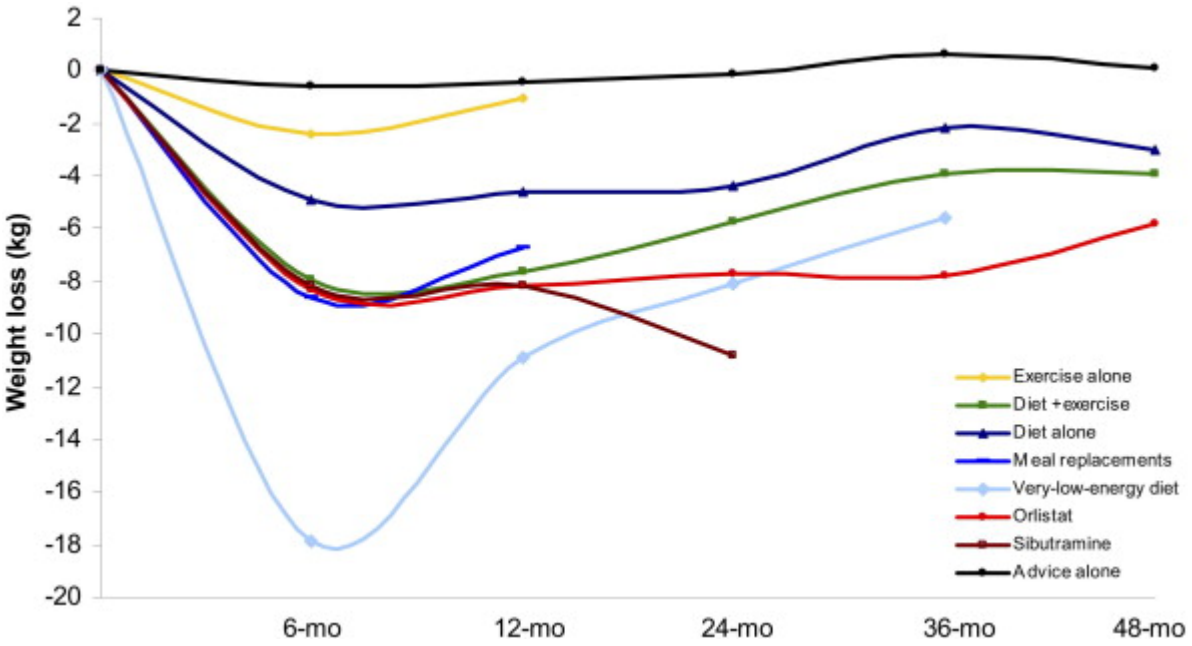


Figure 1.5. Average weight loss of individuals completing a minimum 1-year weight-management intervention; based on review of 80 studies (N=26,455; 18,199 completers [69%]) (Franz et al., 2007).

Challenges to sustaining weight loss have been reported as being largely varied and comprise of returning to old habits enabling weight regain, new dietary interventions not fulfilling needs, such as emotional, social or stress related needs, or struggling to cope with a change in identity as a result of losing weight (Greaves et al., 2017, Jallinoja et al., 2008, Engström and Forsberg, 2011, Green et al., 2009). Furthermore, it has been reported that individuals who have regained weight have described feeling a sense of deprivation, which is derived from feeling like weight maintenance is all or nothing; success or failure. This was described as feeling like they had not achieved weight maintenance if they didn't maintain a set weight target (Byrne et al., 2003). Lack of support from family members has been reported as presenting a significant challenge where attempting weight loss maintenance (Benson-Davies et al., 2013).

Given the reported challenges that individuals may face when attempting to maintain weight loss, it is important that strategies are put in place to best facilitate weight maintenance. For example; involving family members on the individuals needs, such as not introducing temptations. Individuals aiming to maintain weight loss should be counselled on factors affecting sustaining weight loss; such as motivations for maintaining weight loss, implications

on health and establishing an early intervention for preventing weight gain (Avery et al., 2017).

1.4. Very low calorie diet

A very low calorie diet (VLCD) is widely defined as an extremely hypocaloric diet; in practise, VLCDs have been reported as varying between 600-1200kcal/ day (Chen and Yan, 2006, Tsai and Wadden, 2006), delivered from 8-16 weeks (Disease, 2012) and have been achieved using a variety of methods, such as meal replacement products or food based approaches. They have been historically used successfully for weight-loss within specialist settings. When undertaken as instructed, VLCD's provide all of the dietary reference values required for healthy individuals. When compared to specialist- delivered behavioural programmes for weight-loss, behavioural weight-management programmes combined with VLCD's have been shown to elicit a 3.9 kg greater weight-loss after one year (Parretti et al., 2016). However, this review comprises mainly small trials, often including <100 participants and it is therefore likely that this could be inaccurate. Larger scale studies are required in this area to fully evaluate the potential role of VLCD's when compared to other holistic weight-loss approaches.

1.4.1. VLCD and weight loss

Typically, VLCDs are recommended for those with obesity or obesity-related comorbidities (Saris, 2001). As previously mentioned, they are often, but not always (Morris et al., 2020), comprised of meal replacement products. These products contain a low level of carbohydrates combined with a high level of protein to aid satiety and weight-loss. To ensure all vital nutrients are consumed, they also contain vitamin, mineral and fatty acid supplements. The primary goal of a VLCD is to elicit rapid weight-loss, where the majority of weight lost is fat mass, and lean mass is maintained. Subsequent to rapid weight loss, it has been shown that VLCD's can induce other health benefits such as a reduction of blood pressure, improved metabolic control, and reduced liver size and hepatic fat content (Lewis et al., 2006).

A noTable study evaluating the safety and effectiveness of a very low calorie diet as treatment for obesity is the DROPLET study (Jebb et al., 2017). This study was a two arm study delivered through ten primary care practises in Oxfordshire where recruited participants were

randomly allocated to a VLCD intervention or to usual care. This cohort comprised of 278 adults who were obese and seeking support to lose weight. Obesity was defined as a BMI of at least 30 kg/m^2. The VLCD intervention comprises of 810kcals per day, made up of formula food products, administered for 12 weeks, with weekly behavioural support provided. This was followed by a stepwise reduction of the formula products alongside a gradual reintroduction of food-based meals; during this phase, monthly behavioural support was provided. The usual care arm of the study consisted of weight management programmes provided by practise nurses who have been trained to deliver a weight loss intervention through usual primary care. This arm was delivered over twelve weeks, with either weekly or biweekly appointments. Participants within the usual care arm were not prevented from attending other weight management schemes, but these were not provided by NHS referral for the duration of the study. Further analysis of this data reported that individuals from practices located in most deprived and intermediate deprived tertiles were more likely to enrol in the VLCD intervention compared with those in the least deprived tertile (Astbury et al., 2020b). Furthermore, there was no evidence that age, a pre-existing diagnosis of type 2 diabetes or hypertension affected uptake. In the intervention group, 13% of participants were low engagers, 8% engaged with the weight loss phase only, and 79% engaged in both weight loss and weight maintenance phases of the programme, with those engaging in the entire programme losing the most weight.

Recently, a VLCD (DiRECT study) has been shown to be successful in overweight (BMI of 27-45 kg/m^2) participants with T2DM not treated by exogenous insulin (Leslie et al., 2016), with 24% of participants allocated to the intervention arm achieving a weight-loss of greater than 15 kg, and remission of T2DM achieved in 46% participants in the intervention group, compared to 4% within the control, at 12 months (Lean et al., 2018). The DiRECT study was a cluster-randomised study, with GP surgeries randomly allocated to intervention or control groups using a computer. The intervention group had weight loss induced using a total diet replacement phase using a low energy formula diet (825–853 kcal/day; 59% carbohydrate, 13% fat, 26% protein, 2% fiber) for 3 months (extendable up to 5 months if wished by participant), followed by structured food reintroduction of 2–8 weeks (about 50% carbohydrate, 35% total fat, and 15% protein), and an ongoing structured programme with

monthly visits for long-term weight loss maintenance. The control group continued to receive standard diabetes care as defined by current guidelines.

Alongside a substantial improvement in diabetes control (as indicated by a reduction of HbA1c and/or withdrawal of prescribed antidiabetic medications), an array of other improvements were reported; reductions of blood pressure and triglyceride levels, and an increase of quality of life. Between 12 and 24 months, mean weight increased by 2.6 kg in the intervention group compared to a decrease of 1.3 kg in the control group, and 36% of the intervention group were still in remission (Lean et al., 2019). From baseline to 12 months follow up, participants lost an average of 10.0 kg (100 to 90 kg, $p<0.001$). HbA1c (mmol/mol) decreased by a mean of 9.6 mmol/mol (60.2 to 50.6 mmol/mol, $p<0.001$). The number of prescribed oral antidiabetics decreased by 0.8 (1.1 to 0.4, $p<0.001$). Retrospective analyses indicated that weight loss was strongest predictor of diabetes remission at 12 months from baseline, and that being prescribed fewer antidiabetes medications, having lower triglyceride and gamma-glutamyl transferase levels also predicted remission to a lesser extent. Furthermore, patients reporting better quality of life with less anxiety/depression was predictive of diabetes remission, with the prescription of antidepressants predicting non-remission. Interestingly, lower HbA1c at baseline was a predictor of remission at 12 months, and older age and male gender were identified as predictors at 24 months (Thom et al., 2020a). Interestingly, a recent retrospective economic evaluation of the data obtained from the DiRECT study (Lean et al., 2019) has shown that the cost of the VLCD intervention led to a significant cost-saving of £120 and £14 for oral anti-diabetes drugs and anti-hypertensive medications, per participant respectively, compared with the control (Xin et al., 2019). This suggests that a VLCD is a feasible intervention to achieve significant weight loss in order to achieve remission of diabetes, and is less resource intensive than the cost of managing diabetes and its' associated complications.

More recently, the feasibility of delivering a food-based approach VLCD within primary care has been assessed for patients with type 2 diabetes (Morris et al., 2020). When compared to the control arm, patients in the intervention group experienced a significant reduction of weight and HbA1c. Importantly, the outcomes from this study suggest that it is feasible to recruit participants to a food-based, low-energy intervention, for practice nurses to deliver the programme in primary care, and to retain participants within the intervention (Morris et

al., 2020). While a meal replacement VLCD has been shown to be effective to elicit significant weight loss in those who elect to take part, it is important to consider that the strict intervention does not necessarily have a large rate of uptake. For example, within the DiRECT study, only 20% of patients who were eligible and invited to take part in the intervention did so. Therefore, the success of this intervention should be treated with caution, as this does not depict a significant effect in the context of the entire population who are eligible. Subsequently, it is important to explore other lifestyle related avenues which may promote a similar physiological effect on weight while observing an improved uptake. Potentially, a food-based VLCD could do so.

1.4.2. VLCD and Quality of Life

There has been conflicting literature on the effect that a VLCD intervention can have on quality of life (QOL) (Ein et al., 2019) and this is a subject of ongoing debate. QOL is an essential measure, as there is a fundamental link between physical and psychological health (Chida and Steptoe, 2008). Symptoms of depression (Beck et al., 1961) and anxiety (Ferreira and Murray, 1983) are common in individuals who are overweight or obese (Carey et al., 2014, Grundy et al., 2014) and therefore are commonly measured in weight-loss or lifestyle interventions. Research indicates that depressive and anxiety related symptoms have been shown to improve after weight-loss surgery or weight-loss programmes (Sysko et al., 2012, Lasikiewicz et al., 2014). Given the diverse nature of VLCDs, it is no surprise that a VLCD programme has been shown to both increase (Buffenstein et al., 2000) and decrease (Snel et al., 2012) depressive symptoms. As aforementioned, VLCD programmes vary immensely, in that some may include low-intensity exercise alongside the diet, or behavioural therapy or even the duration of the programme itself. VLCD programmes are used in obese or overweight individuals, who are likely to have a range of comorbidities, which could also affect pre- and post-programme depressive and anxiety symptoms. Similarly, given the varying duration of a VLCD programme, the varying subsequent weight lost is likely to affect how an individual self-reports on depressive and anxiety symptoms.

1.4.3. Very low calorie diet: guidelines

VLCDs are often anecdotally perceived as being unacceptable to a significant proportion of the population, unsafe and leading to rapid weight regain. However, with regards to guidelines, the National Institute for Health and Care Excellence (NICE) has recommended that VLCDs can be used for a maximum of 12 weeks in populations who would benefit from rapid weight-loss, such as those who need orthopaedic surgery. With regards to weight loss, there are a plethora of recommendations in place that advocate weight-losses of 5% in order to see clinically meaningful improvements in blood pressure, diabetes control (HbA1c and blood glucose), blood lipids and cholesterol and overall CVD risk (Jensen and Ryan, 2014). Behavioural therapy is also recommended to accompany weight-loss interventions to facilitate self-monitoring of food intake to support weight-loss and weight maintenance.

More recently, the cost effectiveness of doctor referral to a commercially provided VLCD weight loss programme, compared to nurse-led behavioural support has been evaluated (Kent et al., 2019). Initial costs per person for the commercially available VLCD and for the nurse-led behavioural support were £796 and £34, respectively. The VLCD was projected as being more cost effective in obese adults, and cost effectiveness did not significantly vary by gender. In the scenario that all weight lost during the intervention was regained by 5 years from baseline, the intervention was still considered to be more cost effective than nurse-led behavioural therapy, due to anticipated future reductions in expenditure (Kent et al., 2019). Maximal cost effectiveness was observed in adults with a BMI > 40 kg/m^2. Given the demonstrated potential cost effectiveness of a doctor referral to a commercial VLCD, it is likely that this approach for inducing weight loss could be successfully incorporated into routine care (Kent et al., 2019).

1.4.4. Very low calorie diet and NAFLD

1.4.4.1. VLCD and NAFLD Physiology

Detailed metabolic analyses were performed in a small subgroup (n=45) of the DiRECT cohort, indicating that immediately post-VLCD, liver fat decreased significantly compared to baseline levels (16.0% ± 1.3% to 3.1% ± 0.5%, $p < 0.001$) (Lean et al., 2018), as indicated in Figure 1.6. Importantly in the remission of early T2DM (<6 years since diagnosis), a VLCD has been shown to lower hepatic acetyl-CoA, glycogenolysis, and TAG-DAG-PKCε activation (Lean et al., 2018). Similarly, a VLCD has been shown to markedly improve hepatic, not peripheral, insulin

sensitivity in rats with T2DM (Perry et al., 2018). Furthermore, the same research showed a VLCD resulted in reductions in hepatic triglyceride and diacylglycerol content and PKCε translocation, associated with improved hepatic insulin sensitivity. Overall, this has a combined effect of reducing hepatic glycogenolysis, acetyl-CoA content, and gluconeogenesis. While this is not specific to a NAFLD cohort, there is a significant overlap between populations and this mechanism has been shown to play a significant role in reducing hyperglycemia, and potentially could play a role in the improvement of NAFLD. A VLCD has also been shown to normalise beta cell function subsequent to reduced pancreas and liver triaglycerol content (Lim et al., 2011).

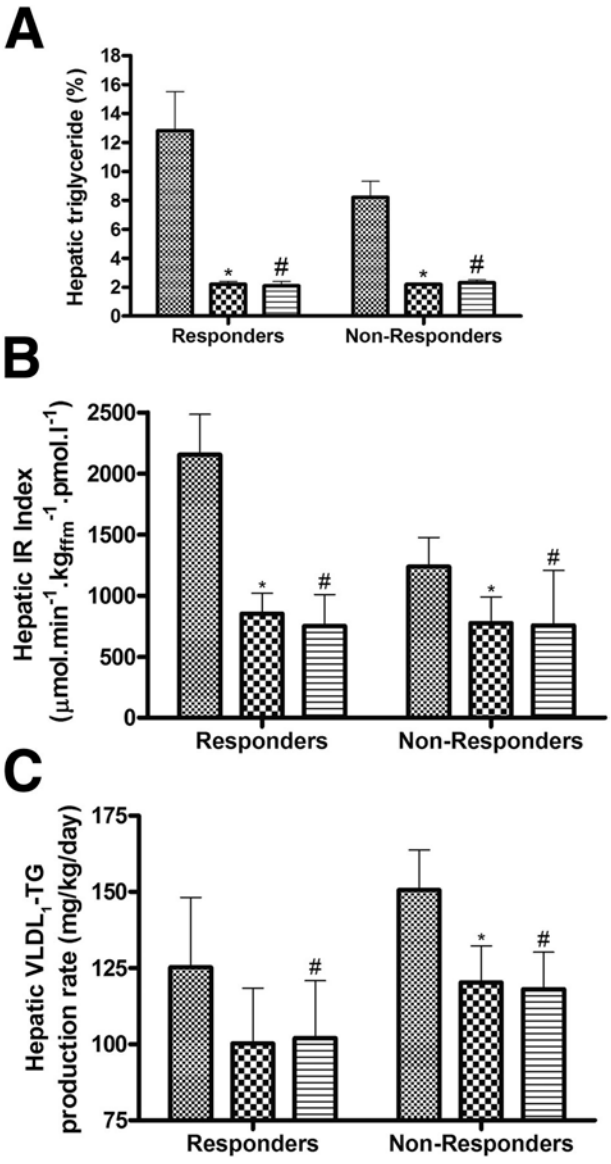


Figure 1.6. The changes in hepatic fat content in responders to a VLCD and non-responders, in a T2DM population (Steven et al., 2016) at baseline (hatched bars), after VLCD (checkered

bars), and after 6 months of weight maintenance (striped bars). Responders were defined as achieving fasting blood glucose <7 mmol/L after return to isocaloric diet. Here it can be seen that responders demonstrated significantly lower levels of hepatic VLDL TG, and overall that a VLCD significantly reduces hepatic fat from baseline.

1.4.4.2. Usage of VLCD within NAFLD to date

It is recommended that weight loss is the primary therapy for NAFLD, and that losses of >10% of baseline bodyweight have been shown to be effective at improving steatosis, inflammation and fibrosis in patients with NASH (Vilar-Gomez et al., 2015). However, there are a plethora of methods advocated in the literature for inducing significant weight loss. Following the widespread success of the DiRECT study (Lean et al., 2018), interest has piqued with regards to the use of VLCD to treat symptoms of the metabolic syndrome.

There is limited research reporting on the use of VLCDs within NAFLD cohorts, not least due to the limitations in conducting research in this population group (see section 1.2.6). A study by Wen-Yuan *et al* investigated the differences between two VLCDs in obese adults (BMI>30 kg/m^2); a 450 kcal/day vs 800 kcal/day regimen (Lin et al., 2009). Abdominal ultrasounds were performed at baseline and at the end of the 12-week intervention (n=93), whereby the baseline ultrasound observed NAFLD to be prevalent in 89% (n=83) of participants. Following the 12-week intervention, NAFLD 'improvement rate' was 42% and 50% in the VLCD 450 kcal/day and 800 kcal/day regimens, respectively. Observed NAFLD was categorized into four subcategories; normal, mild, moderate and severe, as has been previously described in the literature (Saverymuttu et al., 1986). Patients were considered to have improved if they progressed from a more severe subcategory to a category of lesser severity. 16% of all participants with NAFLD returned to 'normal', as defined by abdominal ultrasound (Lin et al., 2009). While Wen-Yuan *et al* did observe improvement in NAFLD, this was not a study specific to a NAFLD cohort. Similarly, ultrasound only detects IHL above 30% so it is difficult to quantify levels of improvement using this method of imaging. There were no other markers of NAFLD reported, such as liver enzymes and subsequent non-invasive scoring methods. Incidentally, patients whose liver enzymes were elevated to double the normal range were excluded from partaking in the study, so it is likely that many with moderate/severe NAFLD were excluded, and this improvement in NAFLD is therefore not representative of a typical NAFLD cohort.

Similarly, patients with T2DM were excluded from partaking. Given the large crossover of T2DM and NAFLD, this is also likely to exclude those with more severe NAFLD.

Another study investigated the prevalence and incidence of simple steatosis and NASH in morbidly obese patients who undertook a VLCD pre-bariatric surgery (Schwenger et al., 2018). 139 patients had biochemical measures taken pre-VLCD and 21 patients had measures taken afterwards. Wedged liver biopsies were taken during the bariatric surgery. NAFLD was diagnosed in 76% of all 139 baseline patients (62% with simple steatosis and 14% with NASH). The remaining 24% had normal livers. Patients with NASH had significantly higher ALT, AST, insulin resistance and proportion of individuals with T2DM than those with normal liver (Schwenger et al., 2018). The VLCD had a median length of 3 weeks with weight loss per week being approximately 3.7 kg. This VLCD induced a significant decrease in BMI, HbA1c, fasting cholesterol, glucose, and insulin, as well as significant increases in AST and ALT. Changes were similar between those with normal liver, simple steatosis and NASH. This study took place in Canada, where VLCDs are routinely administered as part of standard pre- bariatric surgery protocol to reduce the size of the liver in order to facilitate laparoscopic access to perform the surgery (Fris, 2004). Other studies have documented that a minimum 2-week VLCD intervention is sufficient to induce a significant reduction in liver volume (Colles et al., 2006). While a large proportion of the baseline population of this study had NAFLD, it is important to recognize that this is not a NAFLD specific cohort. Similarly, post VLCD data was only available for 21 patients, so the overall comparison dataset for biochemical changes pre- and post- VLCD is small. It is possible that the small dataset means that significant differences between groups were not detected. Similarly, given the relatively short time spent on the VLCD regimen, it is possible that other, 'non-immediate', effects were not able to be observed. It is likely that other histological effects of a VLCD are observed after 3 weeks (Schwenger et al., 2018). While the findings of this study are interesting, they highlight the need for further studies to be done to assess the long term effect of a VLCD on NAFLD.

More recently, a VLCD (600kcal/day) was administered in participants for four weeks, where participants were treated with a prebiotic (inulin) or a similar placebo for the duration of the subsequent weight maintenance phase (Chong et al., 2020). Participants were considered to be engaging in the weight maintenance stage of the intervention until follow up at 28 weeks, with 93% of participants completing the study. The primary clinical endpoint of sustaining

>7% weight loss by week 16 was achieved by approximately 47% of participants, and approximately 34% at 28 weeks. When compared to results from other VLCD studies (Lean et al., 2018), this is a lower level of weight loss; for example, the DiRECT study (Lean et al., 2018) observed a mean percentage weight loss of 10% from baseline weight at 12 months. Given that, at 28 weeks in the aforementioned study, only a minority achieved >7%, it is highly likely that there is a substantial difference in sustained weight loss. This is likely due to the longer duration of the VLCD intervention in DiRECT. This indicates that VLCD interventions can successfully be used in NAFLD cohorts to induce (and sustain) significant weight loss, however, given the short VLCD duration, it is important that further research is undertaken to fully establish how effectively a VLCD can be used in these populations. Additionally, given that it has been shown that VLCDs can be used to successfully induce significant weight gain, it is important to understand potential barriers and facilitators for patients who may potentially partake in a VLCD intervention, to further maximise adherence and subsequent success.

1.4.5. Acceptability of a very low calorie diet

Adherence to VLCDs can be challenging and given the self-report nature of most VLCD interventions, subsequent monitoring of compliance is complex and difficult. Previous trials have reported 'completion' of VLCD interventions between 56%, 70% and 71% (Ryttig et al., 1997, Lee et al., 2010, Moreno et al., 2014), however, this does not necessarily reflect individual participants' adherence to the VLCD protocol. Specifically, following a VLCD in a T2DM population (DiRECT), the completion rate of the VLCD intervention was 83% (Lean et al., 2018). The majority of VLCD-based interventions are delivered as an outpatient service where the monitoring of adherence is likely to be problematic. However, in spite of this, attempts at monitoring the acceptability of VLCDs have been made. The acceptability of a perioperative VLCD in obese individuals has been observed (Colles et al., 2006), where adherence was high initially, but waned throughout the 12-week intervention period. This VLCD intervention used meal replacement products to induce weight loss. As a means of measuring acceptability of the overall intervention 'taste acceptability' was monitored throughout the 12-weeks. It was reported that taste acceptability deteriorated significantly from week 4, and this was significantly positively associated with percentage weight loss, i.e.,

the greater the taste acceptability, the greater the percentage weight loss. Levels of adherence to VLCD interventions in controlled studies is reported to be high in patients with T2DM (Rehackova et al., 2016). For example, when VLCD interventions have been observed alongside comparator arms such as Roux-en-Y gastric bypass cohorts (Lips et al., 2014, Jackness et al., 2013), standard care with behavioral therapy cohorts (Williams et al., 1998), and low energy interventions (Paisey et al., 2002, Wing et al., 1991, Wing et al., 1994), attrition rates of participants did not significantly vary, indicating that a VLCD intervention can be considered to be as acceptable as standard care, the Roux-en-Y gastric bypass pathway and low energy interventions. Primary motivators for continued engagement with VLCD interventions have been identified as experiencing rapid weight loss, increased tangible physical and psychological wellbeing, improvements in blood glucose levels and social support (Rehackova et al., 2017). Techniques employed by participants to maintain adherence included removing food from the immediate environment and planning ahead. Overall, patients reported that the VLCD was easier than originally anticipated but still personally challenging (Rehackova et al., 2017). While this research provides a useful insight into techniques to maximise adherence and acceptability towards a VLCD intervention it is important to remember that this has been observed in T2DM cohorts. Other, less symptomatic clinical cohorts (e.g. people with NAFLD) may be less motivated in losing weight and therefore could be less engaged in personally intense interventions, such as a VLCD.

As part of the DiRECT study (Lean et al., 2018), semi structured interviews were undertaken with each participant at baseline, at the end of the VLCD intervention and at follow-up (Rehackova et al., 2020). Four main themes of change were identified; 'building behavioral autonomy', 'behavioral contagion', 'from rigid to flexible restraint', and a 'shift in identity'. Building behavioral autonomy related to participants ability to develop responsibility for their own health behaviors and becoming more independent when making decisions regarding their health, diet, and lifestyle. 'Behavioral contagion' was defined as a participant changing their behavior, and the people they socialize with changing theirs in turn. Finally, a shift in identity relates to participants desire to become 'healthier people'. Following weight loss, comments from other people, a reduction in clothes size, and the 'status' of being diabetes-free, or medication-free gave individuals a perceived sense of a shift in identity. This is of

paramount importance for the further understanding of how best to induce significant weight loss in overweight individuals with weight-related comorbidities.

Given the conclusive evidence that VLCDs are an effective means of safely inducing significant weight loss and subsequent remission of weight-related comorbidities, the next important step is to evaluate motivators, facilitators and barriers to adherence and engagement. This is an essential stepping stone towards understanding how VLCD's can be made applicable to other clinical cohorts who would benefit.

1.5. Liver Transplant

Ultimately, up to 11% of NAFLD patients will develop end stage liver disease and may require a liver transplant (Anstee et al., 2013b). This demand will increase in the future due to the growing number of people with NAFLD. Within the USA, NAFLD is the fastest growing indication for transplantation, with new waitlist registrants increasing by 170% from 2004 to 2013 (Wong et al., 2015). Currently within the UK, the average time spent on an active waiting list is approximately 145 days for adults awaiting liver transplants and 72 for children. The number of patients on the active transplant list steadily increased from March 2008 to March 2012, with Figures of 268 and 553, respectively. Between 2012 and 2017 that Figure has fluctuated, peaking at 611 in 2015, with the most recent data from March 2017 showing 530 patients on the active waiting list (NHS, 2017). This Figure is inclusive of all seven transplant centres in the UK, and includes all multi-organ, elective and super-urgent registrations. The vast majority of those on the active waiting list are adults, with just 47 paediatric patients of the 530 in 2017. King's College is the largest transplant centre in the UK, with 156 patients on the active waiting list, compared to the smallest centre- Newcastle, which had just 15 patients in 2017. While the average time on the waiting list is approximately 145 days, outcomes for liver-only registrations between April 2014 and March 2015 show that at 6 months post-registration, 51% had been transplanted, 38% were still waiting, 3% had been removed and 8% had died/been removed due to deteriorating condition. At 12 months post registration, 67% had been transplanted, 17% were still waiting, 6% had been removed and 11% had died/ been removed due to deteriorating condition (NHS, 2017).

The severity of liver disease warranting assessment for liver transplantation (LT) is associated with reduced exercise tolerance, aerobic capacity and an overall reduction in fitness (Bernal et al., 2013). Furthermore, anaerobic threshold (AT), as determined by cardiopulmonary exercise testing has been shown to predict survival 1-year post LT (Prentis et al., 2012). Of note, AT is the terminology used to define the greatest level of exercise intensity that can be maintained when glucose utilisation is undertaken using oxygen. Relatedly, Bernal et al. (2013) also showed that those who do not undergo LT benefit from improving AT, where lower AT values and peak fitness (VO_{2peak}) values were associated with non-survivors. This presents a unique and opportune time period to improve fitness levels through lifestyle modifications.

1.5.1. Review of Liver Transplant Guidelines

In light of growing evidence that improved fitness improves post-transplantation outcomes, guidelines now incorporate lifestyle recommendations for promoting maximal pre-LT health, inclusive of diet, fitness, alcohol consumption and smoking status (Burra et al., 2016, Murray and Carithers Jr, 2005) . However, as of yet there is no gold standard or structured implementation of lifestyle changes in clinical practice.

1.5.1.1. Comorbidities associated with those awaiting liver transplant

The primary comorbidities associated with liver disease include, but are not limited to, CVD, metabolic syndrome and T2DM. Following research, guidelines suggest that, in 'absence of significant comorbidities, older recipient age (>70 years) is not a contraindication to LT' (Aduen et al., 2009). Current guidelines for those NAFLD patients awaiting transplant advise that 'comorbidities such as obesity, hypertension, diabetes, and dyslipidaemia need to be assessed and controlled pre- and post- transplant as they increase mortality' ('EASL Clinical Practice Guidelines: Liver transplantation,'); AASLD recommendations include minimising obesity. For example, obese patients (BMI >30) require dietary counselling and a BMI ≥40 is considered a relative contraindication to LT (Martin, 2013). Osteoporosis is highly common in those assessed for LT and it is highly recommended that bone densitometry should be obtained as part of the transplantation evaluation (Martin, 2013). This is particularly important as bone mass is known to diminish in the immediate three months post LT due to high doses of steroids, with risk not returning to pre-LT state until two years following LT

(Collier, 2007). Some studies document osteoporosis frequency as up to 55%, reflecting other risk factors such as physical inactivity and inadequate nutritional status (Collier, 2007).

1.5.1.2. Physical Activity

Despite improved fitness being significantly associated with post-LT outcomes (Prentis et al., 2012), there is limited information available in terms of official recommendation on quantity and vigorousness most beneficial for safely improving/maintaining AT in pre-transplant cohorts. While recommendations are available for management of underlying indications for LT, these do not extend to the immediate pre-transplant period or time on the active waiting list. Furthermore, many patients are assessed for transplant have poor fitness (Prentis et al., 2012) and there are only, at best, vague recommendations of how to improve fitness. In other solid-organ transplantation, namely heart and lung, patients appear to tolerate exercise training pre-transplant, despite the greater number of adverse events compared to other high risk groups (Wallen et al., 2016). Within this meta-analysis, of the 11 studies evaluated, only one reported adverse events due to exercise training in transplant candidates. Despite the ill health of these candidates, improvements were documents in peak work capacity in two studies, with a 43% improvement following an 8-week intervention (Hayes et al., 2012) and a 25% improvement over a 26-week intervention (Ben-Gal et al., 2000). As highlighted by Prentis et al. (2012), this improvement in fitness could result in greater survival in the 90 days following the LT.

1.5.1.3. Diet

Evaluation of nutritional status is an essential tool for pre-transplant assessment. The extensive range of underlying liver disease that may require a transplant means that each individuals' dietary requirements can be widely varied. EASL state that improvement of nutritional status, where appropriate, is indicated, but as of yet no protocols have been approved. In particular, malnutrition is associated with lower post LT survival rate, where patients with BMI< 18.5 are at the greatest risk of poor outcome (Dick André et al., 2009). With regards to weight loss, as previously highlighted, current research has shown that a combination of diet and exercise is the most effective approach. In terms of diet alone, low fat and low carbohydrate diets are gaining popularity within research, however, they are not formally recommended in published guidelines currently. AASLD advocate the reduction of

dietary sodium, with Sharma et al. (2015) showing a correlation between low serum sodium and improved survival post-transplant, where MELD>11 (Sharma et al., 2015), therefore a low sodium diet is becoming more commonly advised as a management approach towards liver disease, both pre- and post-transplant.

1.5.1.4. Alcohol and Tobacco use

It is unanimous between all recommendations that tobacco use should be prohibited in LT candidates, due to the correlation between tobacco consumption and adverse events relating to cardiovascular mortality. For those patients who have been referred for LT evaluation due to ALD, ongoing monitoring is essential for managing abstinence and achieving addiction treatment goals. To allow any addiction issues to be addressed, a minimum period of abstinence of six months is commonly enforced. Additionally, in those who have been admitted due to alcoholic hepatitis or recent alcohol consumption, this may prompt a recovery and thereby forgo the need for a LT (Martin, 2013).

1.5.2. Pre-transplant cohorts

Fundamental to evaluating the pre-transplant cohort is a comprehensive assessment by the clinical team on clinical features, lifestyle, diet, and measures of fitness. While this is all thoroughly assessed as part of the transplant process on a case-by-case basis, it remains unreported on as a full cohort analysis and therefore describing and understanding the population assessed for liver transplant is challenging. Understanding the patient population is also a vital part of designing targeted lifestyle interventions.

The NHS annual report on Liver Transplantation (NHS, 2017) reports on the characteristics of adult elective deceased donor liver transplantations between April 2016 and March 2017, as well as their underlying disease state, which therefore offers some insight into defining the pre-transplant population. This data reports 66% of recipients as male, with a median age and BMI of 56 years and 28 kg/m^2, respectively. Additionally, this data identified 89% of recipients as white ethnicity. There is a vast range of indications for liver transplant, as summarised in Table 1.4, with the most common being alcoholic liver disease (ALD), cancer and Primary Sclerosing Cholangitis (PSC). Table 1.4 also summarises the prevalence of liver disease associated complications, such as ascites, encephalopathy and varices as well as a generic

report on self-reported lifestyle activity. The 2016/2017 annual report also documents the average waiting time on the active waiting list as 86 days. While insightful in its own right, this data does not show the full image, as it does not describe other lifestyle behaviours such as smoking and alcohol consumption status, nor does it define those who are assessed for transplant, but not listed and the reasons they have not been listed on the active waiting list.

Table 1.4: Demographic characteristics of adult elective deceased donor liver transplant recipients, April 2016- March 2017 (adapted from NHS 2017 Annual Report on Liver Transplantation (NHS, 2017))

Indications	**Total (n=706) N (%)**	**Encephalopathy**	**Total (n=706) N (%)**
Cancer	152 (22)	Absent	464 (66)
Hepatitis C	28 (4)	Present	221 (31)
Alcoholic Liver Disease	197 (28)	Not reported	21 (3)
Hepatitis B	12 (2)	**Varices**	
Primary Sclerosing Cholangitis	85 (12)	Absent	242 (34)
Primary Biliary Cirrhosis	58 (8)	Present (not treated)	415 (59)
Autoimmune and Cryptogenic	46 (7)	Present (treated with either shunt or TIPS)	24 (3)
Metabolic	80 (11)	Not reported	25 (4)
Other	47 (7)	**Lifestyle Activity**	
Acute Hepatic Failure	1 (0)	Normal	56 (8)
Ascites		Restricted	248 (35)
Absent	320 (45)	Self-care	322 (46)
Present	371 (53)	Confined	56 (8)
Not reported	15 (2)	Reliant	10 (1)
		Not reported	14 (2)

There are limited reports on modifiable lifestyle patterns pre-transplant, and only few studies investigating the QoL of patients pre-transplant. Furthermore, as with clinical measurements, this is rarely measured in patients who are assessed for transplant but are rejected or deferred. A recent study by Meller et al estimated 60% of post-transplant patients self-report anxiety and depression (Meller et al., 2017). While the psychiatric assessment of those awaiting liver transplant remains incomplete, one study has assessed the relationship between pre-transplant depression and post-transplant survival (Rogal et al., 2016). Rogal et al. found that pre-transplant depression was significantly associated with mortality and longer hospital admissions.

1.5.3. Physical Activity and Exercise Interventions Pre-transplant

Few studies have evaluated the relationship of physical activity in patients awaiting liver transplant or the feasibility of implementing an intervention in clinical practice. However, one recent study from Kings College London has shown that cardiorespiratory fitness levels (VO_{2peak}), as measured using cardiopulmonary exercise testing (CPET) is indicative of 1-year mortality, where higher mortality was associated with lower fitness levels, measured as VO_{2peak} and AT (Bernal *et al.*, 2014). Prior to this, cardiorespiratory fitness was shown to predict post-operative mortality (Prentis et al., 2012), notably an AT of below 9 mL/kg/min has been indicated as a negative predictor of post-transplant survival.

It is well recognised that liver transplantation is associated with hypertension, post-transplant diabetes and dyslipidaemia (Benhamou and Penfornis, 2002), and that 1-year mortality is higher in those who suffered from diabetes pre-transplant (Watt et al., 2010). Therefore, management of these modifiable comorbidities provides an opportune area to provide and assess interventions. NICE guidelines recommend management of comorbidities pre-transplant. However, advice is vague and unstructured and therefore the waiting list provides an opportune time to introduce prehabilitation. Recent studies have demonstrated significant improvements in functional capacity, muscle mass, and self-perceived health status following exercise programmes in patients within a broad spectrum of liver disease (Williams et al., 2015, Román et al., 2016). This effect of exercise is not unique to chronic liver disease- the

functional capacity in a wide variety of other chronic diseases have been shown to improve following an exercise intervention (van der Meer et al., 2012, Smart and Steele, 2011). These include chronic heart failure, COPD and patients requiring dialysis- potential common comorbidities in those with chronic liver disease. Of the exercise interventions trialled in patients with liver disease, more often than not those with end stage cirrhosis have been excluded. Combined with the low numbers in each of the aforementioned studies, further studies are warranted to fully evaluate the safety of exercise interventions in patients with end stage liver disease.

Recently, patients attending the Queen Elizabeth Hospital Birmingham liver transplant unit were recruited to a 12 week home based exercise programme (HBEP) (Williams et al., 2018). This study targeted adults who had been accepted onto the liver transplant waiting list for primary transplant. The HBEP was supported for 6 weeks with telephone support, and therefore offered a unique and safe setting for a HBEP in patients with advanced cirrhosis. A key primary outcome of this study was the assessment of the feasibility of the intervention; this was measured in terms of patient eligibility, adherence, target recruitment and safety (Williams et al., 2019). All criteria of the feasibility related outcomes were satisfied and therefore this study was considered to be suitable. With regards to improvements in aerobic fitness, functional capacity and health related quality of life, measurements were made at baseline, 6 weeks and 12 weeks. Improvements were observed in the incremental shuttle walk test, average daily steps and the short physical battery performance test and health related quality of life (measured using the EuroQol 5-Dimension 5-Level questionnaire)(Williams et al., 2019). While restricted in not offering this intervention to those who have been deferred or rejected from the active waiting list, this was an interesting and novel study evaluating exercise interventions in end stage liver disease populations. More recently, the feasibility of a smartphone application has been assessed for use in patients with end-stage liver disease (Duarte-Rojo et al., 2020). This was reported as being largely successful, with videos, perceived exertion, and motivational features all being widely used by the participants (n=28). 35% of patients observed a significant improvement in physical performance (heart rate-validated steps). Patients reported the sense of community facilitated by the app as a facilitator towards adherence (Duarte-Rojo et al., 2020). This study presents a novel, low cost mode of delivering exercise to patients who were previously largely

sedentary, which, should it prove to be efficacious in a larger cohort, could be well placed to improve the physical fitness of this population.

Overall, a frequently disregarded cohort of patients are those who have been declined transplant. This is a large group of patients who are unable to be transplanted for multiple reasons, including low levels of fitness, and in the future it would be highly valuable to target these patients in terms of lifestyle interventions. Providing an intervention suitable for this cohort would be of huge benefit to them, at a relatively low cost.

1.6. Summary of Literature Review

Liver Disease is the fifth biggest killer in the UK, with NAFLD as the most prevalent aetiology. This unique disease group is growing in prevalence annually, with NAFLD alone thought to affect up to 33% of western populations. NAFLD is directly related to excessive calorie consumption, a sedentary lifestyle and obesity, with a significantly higher incidence in T2DM populations. With no approved pharmaceutical treatment for NAFLD, lifestyle modifications are essential for successful NAFLD management. However, current recommendations for NAFLD cohorts are vague and unstructured on how best to lose weight and increase physical activity levels. Research has highlighted the role of lifestyle within NAFLD management and it is becoming increasingly clear that weight reduction is beneficial in those with NAFLD across the disease spectrum. Even a reduction of body weight by 3% has been shown to improve liver fat and a 10% reduction has been shown to improve hepatic fibrosis. There is currently much debate over the best dietary patterns to follow to maximise weight loss in NAFLD cohorts. Low fat and low carbohydrate have shown promising results in inducing weight loss and allow patients flexibility within their diet. A VLCD has been shown to reduce hepatic fat content and achieve rapid weight loss in both healthy volunteers and patients with T2DM (Steven et al., 2016). As yet, this approach has not been evaluated in NAFLD cohorts, but given the close relationship between NAFLD and T2DM this could be equally as effective.

It is estimated that approximately 40% of NAFLD patients will develop progressive liver fibrosis and ultimately up to 11% will develop end stage liver disease. Due to this increasing epidemic, it is estimated NAFLD will soon be the primary indication for liver transplantation. Once liver disease has progressed to a stage where liver transplantation is a valid treatment

option, often patients have reduced fitness, characterised by reduced exercise tolerance and aerobic capacity. Cardiorespiratory fitness is correlated with post-transplant survival, with an AT of 9 ml/kg/min identified as significant, where values below this are associated with poorer survival post-transplant. Current literature is limited on lifestyle interventions pre-transplant, but it is becoming more widely apparent that the time on the waiting list provides a unique opportunity to improve or maintain fitness. As of yet, there is only one study currently investigating the feasibility of delivering an exercise intervention pre-transplant. This literature review has identified there is minimal information on both the pre-transplant cohorts and the pre-transplant assessment cohorts. The latter group is inclusive of a wide group of patients who could potentially benefit from a fitness intervention to raise their AT to an acceptable level for undergoing transplant.

1.7. Summary of experimental hypotheses and aims

The overarching aim of this thesis is to investigate the relationship between lifestyle interventions and liver disease, with a focus primarily on the feasibility and acceptability of a VLCD in NAFLD. The aims and hypotheses for each of the studies are detailed below:

Chapter 3: The primary aim of this study was to determine whether a minimum 8-week VLCD is a feasible and acceptable therapy to achieve a target weight loss of 10% in patients with clinically significant NAFLD, and whether weight loss could be maintained for at least 6-months following completion of the VLCD.

Chapter 4: This study aimed to initiate and maintain 10% weight loss. Specifically, the qualitative study aimed to identify barriers, facilitators and motivations towards uptake, engagement and adherence to the 8-12 week VLCD (~800kcal/day).

Chapter 5: The primary aim of this study was to objectively evaluate PA levels, inactivity and sleep using 24-hour tri-axial accelerometry in patients with clinically confirmed NAFLD and compare this to age- and gender-matched healthy controls. Furthermore, as an adjunct to the VLCD study (Chapter 3) we aimed to evaluate changes in PA levels and sleep data between pre-VLCD, immediately post-VLCD and at follow up.

Chapter 6: The aim of the study described within this Chapter was to develop an exercise intervention targeted towards patients on the active waiting list to receive a liver transplantation. The development of this intervention was informed by patient feedback throughout.

2. Chapter 2: General Methodology

2.1. Introduction

This thesis is incorporated into two main strands, as shown below in Figure 2.1; evaluating the efficacy and feasibility of a VLCD in NAFLD patients through the use of quantitative and qualitative methodologies and the development of an exercise programme for patients who were undergoing assessment for wait-listing for liver transplantation. The relevant methodologies for both strands are outlined in this Chapter, with further information in Chapters 3-6.

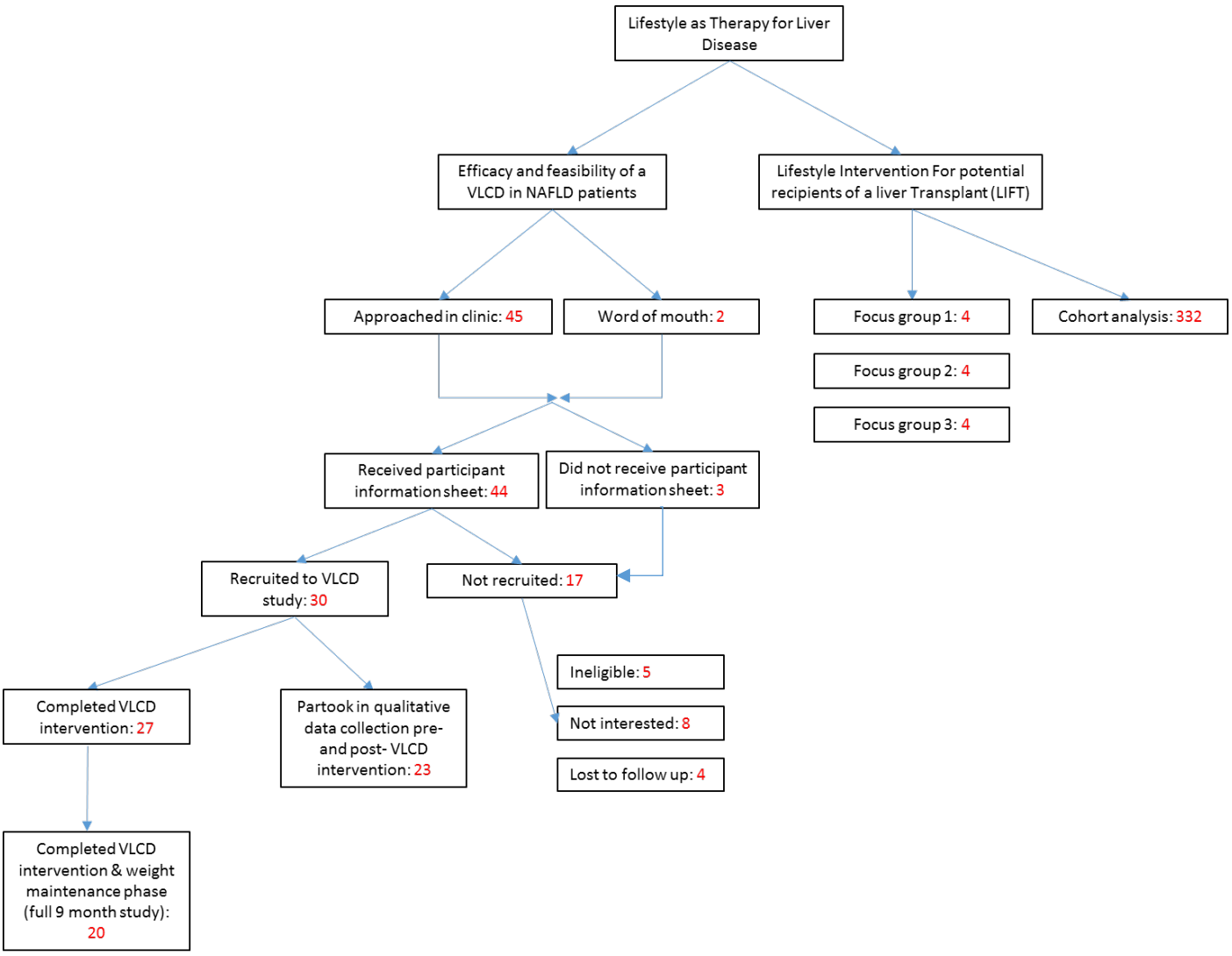


Figure 2.1. Patients contact throughout 'Lifestyle as Therapy' PhD. Red font depicts final sample size for each time point or analyses.

2.1.1. Recruitment strategy

2.1.2. VLCD study

Patients with advanced non-alcoholic fatty liver disease (NAFLD), were recruited from hepatology clinics within the Newcastle upon Tyne Hospitals NHS Foundation Trust from January to July 2019. To facilitate recruitment, clinically significant NAFLD was defined using imaging evidence of steatosis plus an indeterminate or high NAFLD Fibrosis Score (NFS) (≥ -1.455) or Fibrosis-4 (FIB-4) (≥1.3 if age <65; ≥ 2.0 if age ≥65) (Angulo et al., 2007, McPherson et al., 2010, McPherson et al., 2017) or histological evidence of NASH with fibrosis. Potentially eligible patients were identified in clinic by their hepatologist or dietician, and their contact information was obtained and forwarded to a member of the research team. Further information is available in Chapter 3, section 3.2.1.

Data collection commenced on 07/01/2019 and was completed on 17/03/2020, comprising a total of 390 study visit days and approximately 480 hours of face to face patient contact time.

2.2. Informed consent process

Potential recruits were given the relevant patient information sheet (appendix B), study synopsis and information regarding the intervention following referral from hepatology clinics and were given the opportunity to discuss the study with a member of the research team. Following this, a phone call or email from a member of the research team, depending on individual preference, was arranged in order to give them time to digest the information, discuss the intervention with relevant friends and family, and consider any questions that they may have relating to the study. During the follow-up contact, details of the study were explained, potential benefits and risks were discussed, and potential recruits were given further opportunity to ask any questions. If the volunteer was interested in taking part, pre-screening was undertaken and the patient information sheet was provided (appendix B). If they met the criteria, they were invited to attend the first screening/baseline visit at the Freeman Hospital, Newcastle upon Tyne. Family and friends were also invited to attend this visit.

At the screening visit, potential participants were invited to sample the meal replacement products (Optifast, Nestlè Health Science) and to ask any further questions. Patients were made aware that their GP (see appendix D for letter informing the patients GP of their

participation) would be informed about their decision to participate in the study and that they were able to withdraw at any point throughout, without detriment to their future healthcare. If the volunteer was happy to take part in the study, and met the inclusion criteria, they were asked to sign a consent form (see Appendix C).

2.3. Adverse events

At each study visit (described in greater detail in Figure 3.1, Chapter 3), patients were asked if they had experienced any adverse events of side effects of the VLCD since the previous visit. Between study visits, patients were also able to contact a member of the research team to report any adverse events or discuss any concerns that they thought might be related to the study. Similarly, if data obtained at any study visits indicated an adverse event, such a low blood pressure or low fasting plasma glucose in patients with T2DM, this was documented. Patients with T2DM were asked to regularly monitor their blood glucose levels, if they were capable and happy to do so, and discuss any concerns with a member of the research team. Data collection forms were used at every study visit to collate all data collected, which had a subsection for adverse events to be documented. Data collection forms were accordingly added to medical records at the end of each study visit. Should any adverse events have been reported, qualified clinicians within the research team were to be consulted, who were responsible for making any treatment, medication change or referral (for example, to other specialities, such as haematology) decisions and subsequently informing the GP. In line with guidelines specified by the Health Research Authority for a non- Clinical Trial of an Investigational Medicinal Product (CTIMP), a Serious Adverse Events (SAE) protocol was established, whereby any SAE would be reported immediately to the Chief-Investigator who would then contact North East-Newcastle & North Tyneside 1 Research Ethics Committee within 15 days, as the approving ethics committee.

2.4. Qualitative data

Semi-structured one-to-one interviews were used immediately after the VLCD intervention to identify barriers and facilitators to the uptake and subsequent adherence to the VLCD. The interviews were also used to explore ways in which the intervention could be integrated into routine clinical care.

The research team developed a topic guide to structure discussions within the interviews (see appendix E). Topics explored included motivators for taking part; expectations and perceived barriers before participation; barriers to adherence and methods developed to overcome barriers; facilitators and motivators for engagement and adherence; support requirements to maximise adherence to the intervention; and the roles of the patients' social and work environments in maintaining adherence. All questions were open ended and prompts were used to facilitate a more in-depth discussion in order to better explore the patients' views. All interviews were audio recorded and transcribed verbatim. Data were analysed using thematic analysis (Braun and Clarke, 2006).

2.5. Physical examination

Body weight (kg) was measured to the closest 100g and standing height (cm) was measured to the closest 0.1cm using a stadiometer (SECA 799, SECA UK). Both height and weight were performed while the participant was shoeless and body mass index (BMI) was calculated using body weight (kg)/ (height(m))^2. Weight measurements were further standardised by asking all patients to empty the contents of their pockets, remove any outdoor wear or extra layers and encouraging all patients to wear the same, or similar weight, clothing at each study visit. Furthermore, patients attended all visits fasted for at least 8 hours and were encouraged to consistently attend each study visits at the same time of day, where possible. For example, if a patient attended a study visit between 10am and 10:30am, they were asked, if possible, to attend between 10-10:30am at their subsequent study visits. Waist circumference was measured at the mid-point between the lower costal margin and the level of the anterior superior iliac crests. Hip circumference was measured at the level of the greater trochanter. Blood pressure was measured after patients had been seated, at rest, for at least five minutes. Specific measurements were taken on the patients preferred upper arm with legs uncrossed, using a validated Welch Allyn Spot Vital Signs oscillometric noninvasive blood pressure monitor, with either a medium or large adult cuff (Welch Allyn, NY, USA) (Davis et al., 2005).

2.6. Whole Body Composition

Body composition was measured using 8-point Bioelectrical Impedance Analysis methodology. Resistance (R) of arms, legs and torso was measured at frequencies of 1, 2, 5, 10, 50, 100, 200, 500kHz using a seca BIA mBCA 525 machine (SECA 525, Birmingham, UK), pictured in Figure 2.2. This technique has been validated against reference methods for quantifying visceral adipose tissue, such as dual X-ray absorptiometry and magnetic resonance imaging (MRI), with a correlation coefficient of 0.8 reported between BIA and MRI (Kim and Kim, 2013, Bosy-Westphal et al., 2017). The seca BIA mBCA 525 machine was used to collect the data in this study due to its portability and therefore adaptability to be used at the bedside during study visits. Two electrodes were placed on each hand and each foot, which are connected to a small machine that emits an imperceptible current which measures body composition, demonstrated in Figure 2.2. This was performed with the patient lying supine at the baseline, post-VLCD and 9-month follow-up visit by a member of the research team. BIA is based on a two-compartment model that determines total body water (TBW) and soft tissue (i.e. fat mass [FM] and fat-free mass [FFM]). Tissues within the body conduct an electrical current in proportion to their water and electrolyte content. Lean body tissues (high in body fluids and electrolytes), have highly conductive, low resistance electrical pathways. Conversely, skin, bone and fat, are poor conductors and offer high resistance, allowing for differentiation between compartments. Fat mass (kg) and fat free mass (kg), Total body water (%) and visceral fat (kg) were identified as variables of interest.

Body composition measurements were not attempted in those with severe lymphedema or with diabetes related complications of the lower extremities.

Figure 2.2. Measurement of body composition using using a seca BIA mBCA 525 machine (SECA 525, Birmingham, UK).

2.7. Liver Stiffness

Liver stiffness measurement (LSM) was obtained using FibroScan Mini 430 (Echosens, Paris). The Fibroscan procedure uses transient elastography to assess stiffness within the liver. It has been validated in a cohort of 452 patients with NAFLD against liver biopsy, which is considered to be the gold standard for diagnosing and staging fibrosis progression in NAFLD (Boursier et al., 2016). Indeed, in the validation study by Boursier *et* al, Fibroscan was shown to be more accurate than composite scores derived from biochemical evidence for detecting advanced fibrosis, with an area under the receiver operating characteristics score (AUROC) of 0.831 ± 0.019 (Boursier et al., 2016). All patients were fasted for at least 8 hours before the procedure.

The LSM score was represented by the median of 10 measurements and was considered reliable only if at least 10 successful acquisitions were obtained and the IQR-to-median ratio of the 10 acquisitions was ≤0.3 or if the LSM was <7.1 kPa, due to reported lower accuracies in measurements of greater stiffness. Both the medium and extra-large (XL) probes were available for use to determine liver stiffness, which is of particular importance as the XL probe has shown to be significantly more reliable to determine a LSM score in people with obesity (Myers et al., 2012). In a cohort of people with a minimum BMI of 28 kg/m^2, the XL probe obtained a reliable result 73% of the time (Myers et al., 2012). In a study assessing the reliability of the Fibroscan, failure was uncommon (1.6%) and unreliable results occurred in approximately 4.9% of patients, in a sample of 2335 scans in patients with liver disease (Pang et al., 2014). Primary predictors for failure or unreliable results were older age, obesity, higher liver stiffness and operator experience (Pang et al., 2014). To capture the data presented in this thesis, Fibroscan measurements were only undertaken by one of three qualified and experience nurses at study visits.

2.8. Blood samples

A butterfly needle (BD Vacutainer® Safety-Lok™ blood collection system) was inserted into the antecubital or dorsal hand vein using aseptic non touch technique (ANTT) by a trained phlebotomist or qualified member of the research team. Patients were instructed to attend the visits fasted, as well as in a well hydrated condition, to aid in the insertion of the needle. Fasting samples were analysed in a Clinical Pathology Accredited laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry).

2.8.1. Glucose

A 5 ml fluoride/ oxalate vacutainer® of whole blood was drawn at every visit. Samples were analysed using a Roche Cobas C 311 (Kaplan et al., 2003, Moss et al., 1987) automated chemistry analyser to determine glucose levels.

2.8.2. Alkaline Phosphatase

A 5 ml serum separator tube (SST) vacutainer® of whole blood was drawn at every visit. Samples were analysed using a Roche Cobas C 311 automated chemistry analyser (Kaplan et

al., 2003, Moss et al., 1987) to determine alkaline phosphatase levels. Alkaline phosphate levels were determined according to the International Federation of Clinical Chemistry and Laboratory Medicine recommendations (Bermeyer et al., 1986).

2.8.3. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)

A 5 ml SST vacutainer® of whole blood was drawn at every visit. Samples were analysed using a Roche Cobas C 311 automated chemistry analyser (Kaplan et al., 2003, Moss et al., 1987) to determine aspartate aminotransferase and alanine aminotransferase levels. ALT and AST levels without pyridoxal phosphate activation were determined according to International Federation of Clinical Chemistry and Laboratory Medicine recommendations (Bergmeyer et al., 1986).

2.8.4. Gamma-Glutamyl Transferase (GGT)

A 5 ml SST vacutainer® of whole blood was drawn at every visit. Samples were analysed using a Roche Cobas C 311 automated chemistry analyser (Kaplan et al., 2003, Moss et al., 1987) to determine gamma-glutamyl transferase levels. GGT levels were determined according to International Federation of Clinical Chemistry and Laboratory Medicine recommendations (Bergmeyer et al., 1986) and optimally standardised against the original Szasz method (Szasz, 1976).

2.8.5. Full Blood Count

At each visit, a 4 ml ethylenediaminetetraacetic acid (EDTA) vacutainer® of whole blood was drawn and analysed for full blood count. Full blood count was primarily measured in the interest of patients' safety, to further validate any adverse events. However, within the full blood count, variables of interest were platelets as they were required for calculating non-invasive scores- specifically, NAFLD Fibrosis and Fibrosis-4 scores (see sections 2.12.3 and 2.12.4). Samples were analysed using a Sysmex XN-9000 Blood cell processing system. Platelet levels were measured using a DC sheath flow detection method.

2.8.6. Albumin

Albumin was considered to be a variable of interest as it was used to determine a non-invasive score- NAFLD fibrosis score (see section 2.12.3). A 5ml SST vacutainer® of whole blood was drawn at every visit. Samples were analysed using a Roche Cobas C 311 automated chemistry analyser (Kaplan et al., 2003, Moss et al., 1987) to determine albumin levels.

2.8.7. Cholesterol

A 5ml SST vacutainer® of whole blood was drawn at every visit. Samples were analysed using a Roche Cobas C 311 automated chemistry analyser (Moss et al., 1987, Kaplan et al., 2003) to determine cholesterol levels. The Roche cholesterol assay used meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3 % for both precision and bias (Health, 1990).

2.8.8. Triglycerides

A 5ml SST vacutainer® of whole blood was drawn at every visit. Samples were analysed using a Roche Cobas C 311 automated chemistry analyser (Kaplan et al., 2003, Moss et al., 1987) to determine triglyceride levels with an enzymatic colorimetric test principle (Siedel et al., 1993).

2.8.9. Lipoproteins

A 5ml SST vacutainer® of whole blood was drawn at every visit. Samples were analysed using a Roche Cobas C 311 automated chemistry analyser (Kaplan et al., 2003, Moss et al., 1987) to determine Apolipoprotein A-1 (the major protein constituent of high-density lipoproteins (HDL)) and Apolipoprotein B (the major protein constituent of low-density lipoproteins (LDL)). Apolipoproteins were determined using the Immunoturbidimetric assay principle (Siedel et al., 1988).

2.8.10. Insulin

A 5ml serum tube with a silicone-coated interior vacutainer® of whole blood was drawn at three visits; baseline, post-VLCD and at 9 months follow up. Samples were analysed using a solid phase two-site enzyme immunoassay within the Clinical Pathology accredited laboratory (Mercodia Iso-Insulin ELISA). Repeated analysis was required due to large amounts of missing or anomalous data. Repeated analysis of insulin was undertaken in a biosafety level 2 laboratory within Newcastle University using a solid phase two-site enzyme immunoassay (Mercodia Iso-Insulin ELISA).

2.8.11. HbA1c

A 4 ml EDTA vacutainer® was drawn of whole blood at three visits; baseline, post-VLCD and at 9 months follow up. Samples were analysed using a TOSOH G11 analyser to determine glycosylated haemoglobin (HbA1c).

2.9. Measures of deprivation

Deprivation scores were obtained using the Index of Multiple Deprivation (IMD) scoring system (DLTR, 2000). This scoring system uses valid postcodes within England to determine a deprivation score and relative quintile for each postcode. The IMD tool uses third party data from Ordnance Survey Code-Point Open February 2013 and the Office of National Statistic Indices of Multiple Deprivation 2010 to obtain deprivation scores.

2.10. Quality of Life

Patients were asked to fill out the Obesity and Weight-Loss Quality-of-Life (OWLQOL) Instrument (see Appendix F for the full questionnaire) at baseline, post-VLCD, and following 6 months of weight maintenance (Patrick and Bushnell, 2004). This questionnaire gives a quality of life (QOL) score and a weight related symptom measure, and has shown to be reproducible in an obese cohort (intraclass correlation coefficient score >0.95) (Patrick et al., 2004, Patrick and Bushnell, 2004). This QoL tool was used as it has been shown to be responsive to significant weight loss, is brief and easy to complete and has been validated using psychometric analyses (Patrick et al., 2004).

2.10.1. Quality of Life

All 17 OWLQOL items have a 7-point Likert-like response scale, ranging from 0 (Not at all) to 6 (A very great deal). All items are used with equal weighting to derive one single quality of life score. To calculate scores, the numerical response to each item is reversed and all scores are summed, and then the total raw score is transformed to a standardised scale of 0 to 100 using the formula as described in appendix A. Therefore, a score of 0 indicates the greatest impact, and subsequently higher OWLQOL scores indicate a greater quality of life. Patients are allowed to miss up to three items and the score is still able to be analysed.

2.10.2. Weight-Related Symptom Measure

The second half of the questionnaire is a 20 item, self-report measure for prevalence and bothersomeness of weight related symptoms. There is a subset 9 items which specifically

pertain to Weight-Related symptom measure (WRSM) of Diabetes-Related symptoms, and therefore this instrument provides a total WRSM for obese patients, as well as a WRSM for obese patients with diabetes. Patients are asked to identify which symptoms have affected them over the past 4 weeks using 'yes' or 'no' boxes, and are then asked to what degree do the symptoms bother them. The bothersomeness response options are presented as 7 point scale, ranging from 0 (Not at all) to 6 (A very great deal). A total score for bothersomeness is calculated by summing the total of the bothersomeness responses for each item. Total scores range from 0 to 120, with greater symptom burdens indicated by a higher score.

2.11. Physical Activity and Sleep

2.11.1. NAFLD cohort

Free living physical activity and sleep were objectively assessed over seven days at three intervals throughout the intervention- baseline (first week of VLCD intervention), post-VLCD, and following 6 months of weight maintenance. GENEActiv tri-axial accelerometers (ActivInsights Ltd, United Kingdom), as pictured in Figure 2.3, were worn continuously by the patients on either wrist for seven consecutive days at the three time points of measurement. Accelerometers were returned by post and the data downloaded and stored. Patient accelerometer data was only included for further analysis if monitor wear time included at least 3 of the 7 monitored days and at least one weekend day. Previous research has shown that at least three days of accelerometer data are needed to accurately predict PA levels in older adults, compared with four days required when PA is determined by pedometer or a self-report PA log (Hart et al., 2011).

2.11.2. Healthy controls

Healthy controls were age- and gender-matched to the existing baseline NAFLD cohort. Healthy controls were recruited from advertisements around Newcastle University and Newcastle upon Tyne Hospitals. University staff, friends and family expressed interest and following a screening visit informed consent was obtained. Healthy controls then continuously wore the accelerometers for seven consecutive days and they were returned by post. Data was then downloaded and stored, and analysis was only undertaken if individuals

had worn the accelerometer for at least three of the seven days and for at least one weekend day.

Figure 2.3. GENEActiv tri-axial accelerometers (ActivInsights Ltd, United Kingdom)

2.11.3. Analyses of physical activity and sleep data

Raw accelerometer data was processed in R (www.cran.r-project.org) using R-package GGIR (Version 2.0-0) (Van Hees et al., 2013, Migueles et al., 2019, RCore, 2016). Calibration error of the signals were inspected and corrected as described previously (Van Hees et al., 2014). The inclusion of at least 16 hours of valid data for inclusion in the analysis has been described elsewhere (Charman et al., 2016). The average magnitude of wrist acceleration per 5 second epoch was calculated with metric ENMO (1 m*g* = 0.001 x gravitational acceleration) as previously described (Van Hees et al., 2013). Monitor non-wear has been described previously (Van Hees et al., 2013) and was replaced by the average accelerometer data on similar time points on different days of the measurement (Van Hees et al., 2014, Sabia et al., 2014). The imputation procedure has been described elsewhere (Charman et al., 2016). Time spent in

the following acceleration thresholds were calculated: inactivity (<40 mg cut-off) light physical activity (40-100 mg cut-off); moderate physical activity (100-400 mg cut-off), vigorous physical activity (>400 mg cut-off), moderate-vigorous physical activity (≥100 mg cut-off) (Hildebrand et al., 2014, Cassidy et al., 2018). Bouts of moderate-to-vigorous physical activity are identified as all 1 or 5 min time windows that start with a 5 s epoch value equal or higher than 100 mg and for which 80% of subsequent 5 s epoch values are equal to or higher than the 100 mg threshold (Hildebrand et al., 2014).

Estimated total sleep duration (minutes) and sleep efficiency (%) based on absence of change in arm angle greater than 5 degrees for a time period of 5 minutes or longer has previously been described (Van Hees et al., 2015).

2.12. Health risk

2.12.1. Qrisk2

The Qrisk2 score is a means of calculating the risk of a cardiovascular event within the next 10 years, taking into account socioeconomic variables as well as medical variables. Risk factors included self-assigned ethnicity, age, sex, smoking status, systolic blood pressure, total serum cholesterol/ high density lipoprotein cholesterol ratio, body mass index (BMI) and presence of rheumatoid arthritis, chronic renal disease, type 2 diabetes or atrial fibrillation. Additionally, family history of coronary heart disease in a first degree relative under 60 years of age is taken into consideration, as is the prescription of antihypertensive medication and the Townsend deprivation score (Townsend et al., 1988). The output of the Qrisk2 calculation is presented as percentage risk.

2.12.2. Homeostasis model assessment of insulin resistance (HOMA-IR)

The homeostasis model assessment of insulin resistance (HOMA-IR) was used to determine insulin resistance (Bloomgarden, 2006). The HOMA-IR is the product of basal glucose and insulin levels divided by 22.5 and has been widely validated as epidemiological tool (Bonora et al., 2000, Keskin et al., 2005, Matthews et al., 1985). The HOMA of β-cell function (HOMA-B) index is computed as the product of 20 and basal insulin levels divided by the value of basal glucose concentrations minus 3.5 and has been indicated to be a good measure of β-cell

function (Matthews et al., 1985, Wallace et al., 2004). HOMA-S is the inverse of HOMA-IR and allows for the deduction of insulin sensitivity (%) (Levy, 1998).

2.12.3. NAFLD fibrosis Score

The NAFLD Fibrosis score (NFS) (Angulo et al., 2007) is a non-invasive system designed to score and estimate the amount of scarring within the liver. This score is calculated as; -1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×10^9/l) – 0.66 × albumin (g/dl).

A NFS of <-1.455 indicates F0-F2, a score of -1.455-0.675 is considered indeterminate and a score of >0.675 indicates F3-F4.

2.12.4. Fibrosis-4 score

The Fibrosis-4 score (FIB-4) (Vallet-Pichard et al., 2007) is a non-invasive method of estimating the amount of scarring within the liver. FIB-4 scores were calculated as (age(years)x AST(U/L))/(platelets (109/L)x square root(ALT). A FIB-4 score of <1.45 has a negative predictive value of 90% for advanced fibrosis. Alternatively, a FIB-4 score of >3.25 has a predictive value of 65% for advanced fibrosis, with a 97% specificity. The FIB-4 lower cut-off for excluding advanced fibrosis was 1.3 for patients ≤65 years and 2.0 for age >65 (McPherson et al., 2017).

2.13. Statistical analysis

Throughout this thesis IBM SPSS Statistics (version 24, IBM, Armonk NY) software package was used to analyse the data. Statistical analysis that were repeated throughout this thesis are detailed below.

Differences between baseline, post-VLCD and 9 months (Chapters 3 and 5) follow-up were assessed by the use of a one-way ANOVA with Bonferroni post-hoc adjusted pairwise comparisons analysis. Data was assessed for normality and outliers by Shapiro-Wilk test and boxplots, whereupon excessively skewed data was squareroot transformed, logarithmic transformed or assessed by Kruskal-Wallis H test. When the assumption homogeneity of

variances was met, a one-way repeated measures ANOVA was used to determine statistical significance, with significant results interpreted to determine possible group comparisons by Bonferroni post hoc test. Assumption of sphericity was evaluated by Mauchly's test with violated data assessed by Greenhouse-Geisser or Huynh-Feldt. Assumption of homogeneity of variances were assessed by Levene's test for equality of variances. The distributional assumption was used to determine if the Kruskal-Wallis H test was used to compare medians or distribution.

Pearson product-moment or Spearman's rank-order correlation were used to determine the strength and direction of a linear relationship between changes in weight throughout the intervention and changes in other dependent factors (data obtained through the collection of venous blood sampling).

For data analysed in the cohort analysis, data was assessed for normality using the same methodology described above. Where data was normally distributed, an independent t-test was used to compare group differences between waitlisted and non-waitlisted patients. Where data was not normally distributed, a Mann-Whitney U test was used. Crosstabulation was used to assess differences in categorical datasets between groups.

2.14. LIFT study

2.14.1. Recruitment

Data for the cohort analysis of all patients assessed for liver transplantation was obtained through online records and transcriptions recorded from fortnightly transplant assessment meetings where individuals' cases were discussed, from January 2014-May 2017. Based on a range of characteristics, such as general health/wellbeing at the time, ease of attending focus groups and willingness to take part, patients who had been assessed for liver transplantation at the Freeman Hospital were approached and invited to partake in a series of focus groups. Family members were also invited to attend.

2.14.2. Focus groups

The first focus group aimed to capture patients and clinicians' ideas and thoughts regarding an exercise programme, with the purpose of determining facilitators to uptake and adherence, as well as potential barriers that could be avoided. The second focus group aimed

to reflect on themes obtained from the first focus group and gain further insight into factors of acceptability towards the proposed intervention. The third and final focus group aimed to present the preliminary prototype of the intervention and gain feedback in order to further develop it in accordance with patients' views. All focus groups were audio recorded, transcribed verbatim and analysed for recurrent themes and thoughts.

2.14.3. Cohort Analysis

Data was obtained from electronic records and transcripts, and variables of interest were used to compile a database. Data was divided between that of waitlisted and non-waitlisted patients, and also divided by underlying aetiology. Subsequent analyses were undertaken using IBM SPSS Statistics (version 24, IBM, Armonk NY) software package (see section 2.13 for further information).

3 Chapter 3: Feasibility of a very low calorie diet to achieve a sustainable 10% weight loss in patients with non-alcoholic fatty liver disease

Abstract

Objectives: Non-alcoholic fatty liver disease (NAFLD) is the most common liver condition worldwide. A weight loss goal of ≥10% is the recommended treatment for NAFLD, however only a minority of patients achieve this level of weight reduction with standard dietary approaches. This study aimed to determine whether a very low calorie diet (VLCD) is an acceptable and feasible therapy to achieve and maintain a ≥10% weight loss in patients with clinically significant NAFLD.

Methods: Patients with clinically significant NAFLD were recruited to a VLCD (~800 kcal/day) intervention using meal replacement products. Anthropometrics, blood tests (liver and metabolic), liver stiffness and cardiovascular disease risk were measured at baseline, post-VLCD, and at 9-months follow-up.

Results: 45 patients were approached, 30 were enrolled, 27 (90%) completed the VLCD intervention and 20 (67%) were retained at 9-months follow-up. The VLCD demonstrated to be feasible to deliver, and acceptable to patients. Analysis undertaken in all 30 patients, irrespective of completion, found that 34% of patients achieved and sustained ≥10% weight loss, 51% achieved ≥7% weight loss and 68% achieved ≥5% weight loss at 9-months follow-up.

For those completing the VLCD, liver health (liver enzymes and liver stiffness), cardiovascular disease risk (blood pressure and QRISK2), metabolic health (fasting glucose, HbA1c and insulin) and body composition significantly improved post-VLCD and was maintained at 9-months.

Conclusions: VLCD offers a feasible treatment option for some patients with NAFLD to enable a sustainable ≥10%, weight loss, which can improve liver health, cardiovascular risk and quality of life in those completing the intervention.

3.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver condition worldwide, affecting an estimated 20-33% of the population in Western countries (Estes et al., 2018). This condition is directly linked to chronic excess calorie consumption, lack of physical activity/exercise and overweight/obesity. NAFLD is a spectrum of liver disease ranging from isolated fatty liver through to non-alcoholic steatohepatitis (NASH), which can progress to cirrhosis. Dual biopsy studies indicate that approximately 40% of patients with NAFLD develop progressive liver fibrosis (McPherson et al., 2015). Ultimately, 5-11% develop advanced liver disease and have the potential to develop cirrhotic complications (McPherson et al., 2015, Anstee et al., 2013a). As a result, NASH is a common indication for liver transplantation (Bellentani et al., 2010, Shaker et al., 2014). Stage of liver fibrosis is a strong predictor of both liver-related and all-cause mortality in patients with NAFLD (Hagström et al., 2017, Taylor et al., 2020). As such, a therapy that could halt or reverse liver fibrosis may reduce risk of liver-related complications.

In the absence of approved pharmaceutical agents, lifestyle modification, involving weight loss, is the primary recommended therapy for NAFLD (Chalasani et al., 2018, NICE, 2016c), and a weight loss goal of 10% is recommended for patients with advanced NAFLD (Vilar-Gomez et al., 2015, Dyson et al., 2014, Patel et al., 2009). A 2015 study found that 90% of patients losing >10% body weight had resolution of steatohepatitis, and 81% showed improvement in fibrosis (Vilar-Gomez et al., 2015). However, only 10% of those patients maintained 10% weight loss at one year. A randomised controlled trial assessing the effect of weight loss on NASH (Promrat et al., 2010) reported a relationship between percent weight loss and improvement in NAFLD activity score (NAS). Patients who achieved weight loss of >7% had significant histological improvements in steatosis, lobular inflammation, ballooning injury and NAS when compared to those losing <7%. No change in fibrosis scores were reported, and mean weight loss in the intervention arm was 9.3%. These studies highlight the need for acceptable alternative interventions to elicit sustained weight loss of greater magnitude in a larger proportion of individuals.

Very low calorie diets (VLCDs) have demonstrated to be a viable treatment strategy for people with type 2 diabetes mellitus (T2DM) (Steven et al., 2016). Research has shown that VLCDs are effective for achieving substantial weight loss, with high levels of adherence and low levels

of attrition in overweight and obese people with T2DM (Rehackova et al., 2016). A large randomised controlled trial of VLCD (DiRECT) conducted in primary care involving patients with T2DM found that 24% of those in the intervention group lost ≥15 kg, and mean body weight fell by 10 kg at one year follow-up (Lean et al., 2018). As well as this study reporting sustained weight loss, 46% of patients had normalisation of blood glucose control at one year. More recently, results derived from a 2 year follow up have been published; at 24 months within the intervention arm, 11% of patients had sustained a weight loss of 15 kg or more, compared to 2% from control arm. Within the intervention arm, 36% of patients had sustained diabetes remission, compared to 3% of the control patients (Lean et al., 2019). Another study showed that 45% of obese patients undertaking a 12-week VLCD maintained ≥10% weight loss at one year follow-up (Jebb et al., 2017). Research suggests that VLCD may also have a positive impact on fatty liver. One of the first VLCD studies in 11 patients with T2DM (Lim et al., 2011) found that individuals treated with VLCD had a reduction in liver fat (measured by MR spectroscopy) from 13% to 3%. Despite these findings, the VLCD approach has not been formally assessed as a treatment strategy for NAFLD. The totality of these changes could be beneficial to patients with NAFLD in reversing liver disease or halting disease progression, and reducing other obesity-related risk factors.

The primary aim of this study was to determine whether a minimum 8-week VLCD is a feasible and acceptable therapy to achieve a target weight loss of 10% in patients with clinically significant NAFLD, and whether weight loss could be maintained for at least 6-months following completion of the VLCD. Secondary outcome data were collected to characterise the effects of VLCD upon factors that influence the development and progression of NAFLD. This study hypothesised that a VLCD intervention would be feasible to recruit to and acceptable to patients with advanced NAFLD. Furthermore, this study hypothesised that it is feasible for some patients with advanced NAFLD to achieve a significant weight loss of 10% of their initial starting body weight following an 8-12 week 800 kcal/day intervention.

3.2 Patients and Methods

3.2.1 Study design and sample size

This single-arm, single-centre feasibility study aimed to establish whether it is feasible to recruit patients with advanced NAFLD to partake in a VLCD and retain them for the duration of the study. Furthermore, this study aimed to assess if this intervention is acceptable to people with advanced NAFLD and if it is people for the recruited patients to achieve a significant weight loss of 10% of their initial body weight. Therefore, an embedded qualitative study (Chapter 4) was incorporated to capture the views of patients and explore barriers and facilitators towards enrolment and adherence. This study did aim to compare the efficacy of a VLCD to other current treatments or care pathways within standard care, therefore a single arm was considered sufficient to inform the feasibility of recruiting to the intervention. The results of this study are intended to inform if progression to a larger scale study might be suitable, in order to determine the efficacy of a VLCD to achieve 10% weight loss and improve parameters of liver health. While formal progression criteria weren't predetermined, data obtained from this study could be useful in order to inform a power calculation to potentially determine the sample size of a larger study to ensure adequate statistical power when assessing efficacy to achieve significant weight loss and/or improvements in markers of liver health.

A target sample size of 30 was determined in line with previous literature (Lancaster et al., 2004, Browne, 1995). However, other literature is conflicting and suggests a sample size of up to 50 for a feasibility trial (Sim and Lewis, 2012). While a larger sample size may confer greater confidence in any onwards power calculation or the acceptability of the VLCD, in this instance it was critical to work within the constraints imposed by funding limitations and the time scale of this PhD project. Overall, it was judged that a target sample size of 30 would be adequate to inform feasibility to recruit to a perceived drastic intervention such as a VLCD and subsequent acceptability.

3.2.2. Recruitment and patients

Forty five patients with a diagnosis of clinically significant NAFLD and a BMI > 27 kg/m^2 were approached to take part in the study. Thirty patients agreed and were subsequently recruited from hepatology clinics within the Newcastle upon Tyne Hospitals NHS Foundation Trust (NuTH) from January to July 2019.

3.2.3. Inclusion criteria

To facilitate recruitment, clinically significant NAFLD was defined using imaging evidence of steatosis plus an indeterminate or high NAFLD Fibrosis Score (≥ -1.455) or FIB-4 (≥1.3 if age <65; ≥ 2.0 if age ≥65) (Angulo et al., 2007, McPherson et al., 2010, McPherson et al., 2017), or histological evidence of NASH with fibrosis. By including patients with "indeterminate/high risk" NAFLD without a liver biopsy, the pool of eligible patients was substantially increased and this also meant that the results of the study were applicable to a wider NAFLD population. Patients with compensated NASH cirrhosis (Child-Pugh score <7) were also eligible to participate. Other inclusion criteria specified age ≥18 years, weight stability (+/-3%) since biopsy/non-invasive assessment of liver health and capacity to provide informed consent.

3.2.4. Exclusion criteria

Patients were excluded if they had evidence of co-existing liver disease (e.g. autoimmune liver disease, viral hepatitis, alpha-1 anti-trypsin deficiency, haemochomatosis or Wilson's disease), decompensated NASH cirrhosis (Child Pugh score ≥ 7), current treatment with anti-obesity drugs, a diagnosed/previous eating disorder or purging, excessive alcohol consumption (>21 units/week for males; >14 units/week for females), insulin use to manage T2DM, known cancer, myocardial infarction within six months, and pregnant/considering pregnancy. Subject characteristics can be found in Table 3.3.

3.2.5. Ethical approval

The study protocol was approved by North East-Newcastle & North Tyneside 1 Research Ethics Committee (REC reference: 18/NE/0179). This study was retrospectively registered to the ISRCTN registry (ISRCTN Register number: ISRCTN85177264, date assigned to register: 08/08/2019). While this study was registered after closure of recruitment and during data collection, it was registered prior to data analysis. All patients provided written informed consent. Following withdrawal from the study, patients were no longer followed up by the research team and usual clinical care continued. Data was collected and analysed up until their most recent visit.

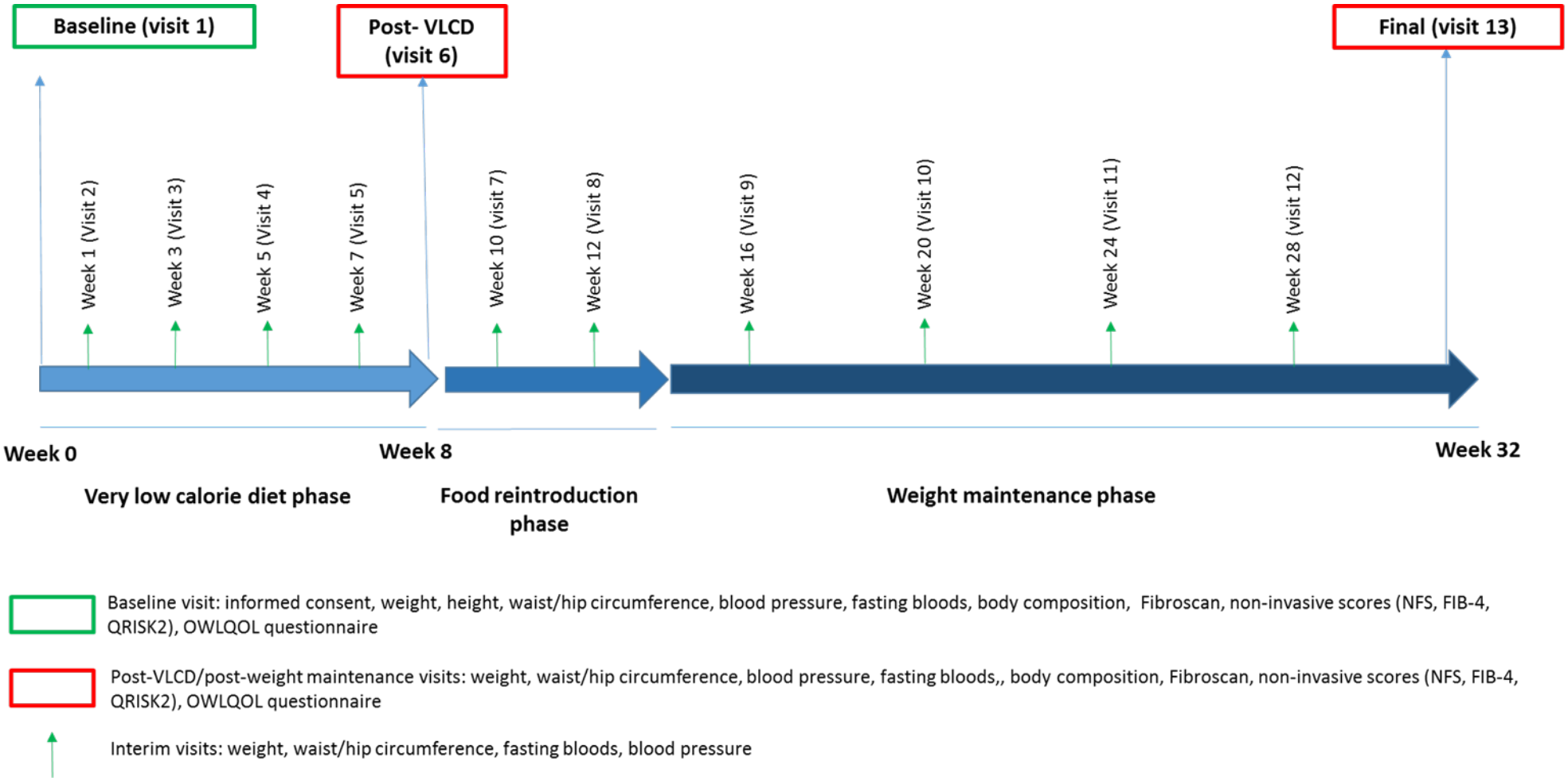


Figure 3.1. Summary of the study schedule and highlights the investigations completed at each visit

3.2.6. Study outcomes

Primary outcomes: Feasibility and acceptability of the VLCD, including feasibility of recruitment, retention and delivering the VLCD, and acceptability of the VLCD to patients and percentage of patients achieving ≥10% weight loss and sustaining it for at least 6-months following completion of the VLCD intervention.

Secondary outcomes: Absolute change in body weight; change in clinical blood markers; change in cardiac (QRISK2/blood pressure/lipids) and T2DM risk (HbA1c/HOMA-IR/glucose/medication changes); and quality of life (all measured post-VLCD and at 9-months).

3.2.7. Anthropometry

Body weight (kg) and height (cm) were measured using an electronic stadiometer (SECA 799, SECA UK). In those lost to follow-up, weight was measured at their next routine clinic visit as per standard care, the majority within 8-weeks of their planned final study visit. Waist circumference was measured at the mid-point between the lower costal margin and the level of the anterior superior iliac crests. Hip circumference was measured at the level of the greater trochanter. Body composition was measured using 8-point Bioelectrical Impedance Analysis (SECA BIA mBCA 525 machine, SECA, UK).

3.2.8. Blood samples

Fasting samples were analysed in a Clinical Pathology Accredited laboratory (Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry) for: Liver enzymes (including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT)), fasting glucose, HbA1c, insulin, lipid profile and full blood count (FBC). See section 2.8 for a more detailed description.

3.2.9. Liver stiffness

Liver stiffness measurement (LSM) was obtained using FibroScan Mini 430 (Echosens, Paris). All patients were fasted for at least 8h before the procedure. The LSM score was represented by the median of 10 measurements and was considered reliable only if at least 10 successful acquisitions were obtained and the IQR-to-median ratio of the 10 acquisitions was ≤0.3 or if the LSM was <7.1kPa.

3.2.10. Non-invasive risk scores

The NAFLD Fibrosis Score (NFS) (Angulo et al., 2007) and Fibrosis-4 (FIB-4) Score (Sterling et al., 2006) - validated non-invasive systems to diagnose or exclude advanced liver fibrosis, were calculated from blood tests at clinic visits. The QRISK2 (Hippisley-Cox et al., 2008) was calculated to estimate the risk of an individual having a cardiovascular event within the next 10 years. The homeostasis model assessment of insulin resistance (HOMA-IR) was used to determine insulin resistance (Bloomgarden, 2006) (see Chapter 2.12). All were calculated for each patient at baseline, post-VLCD and at 9-months follow-up.

3.2.11. Quality of life

Patients completed the Obesity and Weight-Loss Quality-of-Life (OWLQOL) Instrument (Patrick and Bushnell, 2004) which gives a Quality of Life (QOL) score (17 item) and a Weight-Related Symptom Measure (WRSM) (20 item). Lower scores in the QOL section indicate a poorer QOL; higher scores in the WRSM section indicate greater symptom burden (see Chapter 2.10).

3.3. Intervention protocol

Figure 3.1 outlines the structure of the study intervention, as described throughout the following section. See Appendix G for a detailed description of data collected at each time point.

3.3.1. VLCD Intervention

Patients were prescribed an 8-week VLCD (~800 kcal/day) intervention. In the event that consistent compliance with the diet was not possible throughout the 8-week period due to external factors (e.g. hospital admissions or travel), the intervention was extended for an additional four weeks, to a maximum VLCD intervention of 12 weeks. Following completion, patients moved on to the food-reintroduction phase of the intervention.

The VLCD intervention was supervised by a member of the Research Team and patients were provided with meal replacement products (Optifast, Nestlè Health Science. Nutritional content: fat 19.4%kcal; carbohydrate 43.4%kcal; fibre 3.5%kcal; protein 33.7%kcal) free of charge (see Figure 3.2 for the range of flavours available to patients). In addition, patients were encouraged to eat three portions (240 g) of non-starchy vegetables and drink at least two litres of water or calorie-free beverages each day (Appendix H: Newcastle Diet Plan) and were permitted up to 100 ml of semi skimmed milk per day in hot beverages (see Figure 3.3). See Appendix I for a full nutritional breakdown of each of the Optifast products used within the VLCD. One-to-one support was provided weekly throughout the VLCD phase by a tailored combination of phone calls, emails and face-to-face appointments to maximise adherence to the protocol and to minimise drop out. Patients were provided with scales to weigh themselves at home if needed**.** Dietary compliance was monitored by change in body weight. Patients were asked to maintain their usual physical activities during the VLCD but not to increase their activity levels during this phase.

Figure 3.2. Optifast flavours available to patients

Figure 3.3. Composition of 800kcal/day VLCD

3.3.2. Food reintroduction

Following completion of the VLCD phase, patients were supported to follow a stepped return to normal eating over a 4-week time period (described in Figure 3.4). This involved replacing one meal replacement product with normal food in the first two weeks, with education on portion size using the "Carb and Calorie Counter" manual (Cheyette, 2010). Two normal meals were introduced during weeks 3 and 4. If desired, this phase was extended to 6-weeks to help manage individual needs. Specific individualised dietary advice was provided using a food exchange model. The goal was to limit energy intake to individual requirements to maintain weight and patients received support to overcome behavioural barriers (e.g., resisting temptation). Patients were advised to monitor their weight weekly at home and were encouraged to monitor their caloric intake. Each patient was provided with two resource books which contained low calorie meal plans, recipes and snack ideas (Cheyette, 2017), and information relating to the portion sizes and nutritional value (calories, protein, fat, carbohydrate and fibre) of common foods (Cheyette, 2010). Patients were encouraged to increase their physical activity levels during food introduction, and pedometers (Omron

Walking Style One 2.0, Omron Healthcare UK Ltd) were provided for self-monitoring of daily step count. If appropriate, patients were referred to local "Exercise on Referral Schemes" for more structured exercise programmes (seven of our cohort were referred). To allow flexibility for patients, the food reintroduction phase could be between 2-6 weeks, depending on the individual's confidence in themselves to not rapidly regain weight by returning to previous dietary habits. Patients were asked to attend fortnightly visits during this stage of the intervention, shown in Figure 3.1.

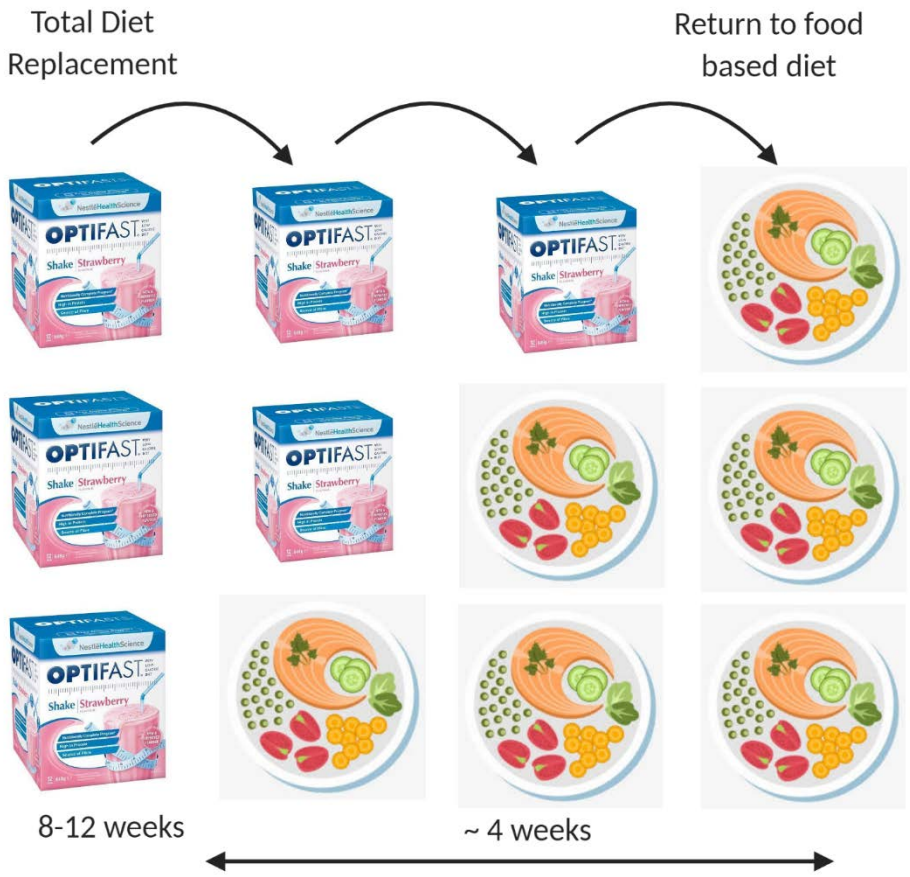


Figure 3.4. Process of stepped food reintroduction from VLCD to food-based diet, with the first column depicting 3 meal replacement products a day and a gradual increase of food based meals throughout the following columns.

3.3.3. Weight maintenance

As part of the final phase of the intervention, patients were advised to follow a food-based diet and were provided with an individually tailored energy prescription, in order to prevent weight regain and support weight stabilisation and/ or further weight loss. Patients were also given the option of using one sachet of liquid formula diet per day. Review appointments were at approximately monthly intervals, where weight, blood pressure, waist and hip circumference were monitored and fasting bloods are taken (liver enzymes, glucose, lipid profile and full blood count), as per Figure 3.1.

Those who were physically capable were advised to increase their daily physical activity or exercise. Pedometers were provided to those who were interested in monitoring their daily step count, in order to allow patients to monitor and increase their daily physical activity.

During the follow-up period, if patients experienced weight regain, they were able to restart the VLCD for 2-4 weeks, with fortnightly reviews during this time, as per the protocol for the initial VLCD (n=3). Alongside the VLCD, support for weight regain included an exploration into the reasons for weight regain, in order to provide support to prevent recurrence.

Justification for intervention

An 8-12 week VLCD intervention comprising of meal replacement products was chosen as previous research in similar populations has used a similarly structured intervention (Astbury et al., 2018, Lean et al., 2018). Specifically, in populations with obesity (DROPLET study) and with T2DM (DiRECT). Given the underlying pathophysiology of NAFLD (Chapter 1, Section 1.2.2), there is likely significant overlap in patient demographics between a NAFLD cohort and cohorts of T2DM and obesity. In the aforementioned previous literature, the VLCD has been undertaken with minimal adverse events, and therefore was judged that it would be suitable safe for the NAFLD cohort. Furthermore, a VLCD was chosen over other dietary interventions due to its highly structured nature, which leaves less room for misunderstanding what to eat compared to other food based dietary interventions. A primary outcome of this study was to determine the number of people to achieve 10% weight loss, and the 8-12 week 800 kcal/day intervention has previously been shown to be efficacious to achieve such a significant weight loss in other obese cohorts (Lean et al., 2018, Astbury et al., 2018). Importantly, a research dietician from the DiRECT study provided training to the members of the research team of

this study who would be delivering the intervention, which was considered beneficial as this likely improved the fidelity and faithfulness of the delivery of the VLCD intervention. This overall ensured that those who delivered the intervention had all received the same training and could deliver it in a more reproducible manner than if an intervention without training had been used. Briefly, training on how to deliver the intervention covered key behaviour change techniques that have been successfully employed to provide support to patients with T2DM in the DiRECT study and therefore informed the behavioural support to be provided to patients in this study.

3.3.4. Changes to medication and safety measures

Sulphonylurea oral hypoglycaemic agents (Gliclazide, Glimepiride, Tolbutamide) were withdrawn on commencing the VLCD, as per the study protocol. Any other diabetic medication was continued as normal throughout the study unless specifically instructed by a member of the research team and regular glucose monitoring was undertaken. Blood pressure was monitored regularly as part of the study protocol, and adjustments made to blood pressure lowering medications made as required. All other medications were continued as usual. Any changes to medication were made by a qualified member of the research team and the patient's GP informed.

Blood pressure, fasting glucose and symptoms of postural hypotension were monitored at each visit. In the event of symptoms relating to these measures, medications were adjusted accordingly by a qualified member of the research team, if necessary, using standard protocols under national clinical guidelines (Appendix J: Protocols for management of inadequate blood glucose control and blood pressure). Enquiries at each visit were made to identify the occurrence of adverse events, and were recorded. Observation and results which posed a potential health risk were identified, discussed with the patient and reported to their GP with the patient's consent via letter/phone call.

If patients dropped out of the study who had had their medication altered as part of the VLCD intervention, the patient was advised to make an appointment with their GP to discuss reviewing/resuming their medication needs.

3.4. Data analysis

All primary and secondary data analyses were performed using IBM SPSS version 24 (IBM, NY, US). Continuous data were tested for normality using the Shapiro-Wilk test and data are presented as means ± SD, unless otherwise stated. Within-group changes were assessed by repeat measure one-way ANOVA, or by Kruskal-Wallis analysis where data was non-parametrically distributed. P-values <0.05 were considered statistically significant. Correlations were measured using a Pearson Correlation Coefficient. Overall p-value in Table 3.3 represents results derived from one-way ANOVA, with further significance explored using a Bonferonni corrected post-hoc analysis. Data for the primary endpoint and overall weight loss outcomes were analysed using the data from all participants, irrespective of completing the intervention to 9 months. Where data was unavailable for weight for this analysis, patients were contacted and asked to provide an up to date weight. If this was not possible, weight recorded at their most recent study visit or general hepatology clinic visit was used, as agreed with patients at the time of enrolment. Henceforth throughout this this, analyses where all 30 patients have been included will be referred to as 'irrespective of completion' (IOC) analyses. The analysis that was conducted to assess the changes in clinical parameters used patients with full datasets across the 9 month intervention, as is required for analysis by ANOVA. No attempts were made to analyse the data sets where there was missing data, as the data was not missing at random (MAR) and therefore could not be accurately fitted to any predictive mixed models or accounted for using missing data imputation.

3.5. Results

3.5.1. Primary outcomes

The primary outcomes of this study were feasibility to recruit to and deliver the intervention, patient acceptability of the intervention and achievement of 10% weight loss at follow-up. This study was fully recruited at a single site within six months, indicating feasibility to recruit to the sample size target. Of the 45 patients approached to take part in this study, 30 (67%) consented to enrol. Overall, 27 (90%) patients completed the VLCD phase of the intervention (16 patients completed 8-weeks of VLCD; 11 completed 8-weeks plus the optional 4-week extension period). Of these, 20 (67%) remained in the study to the end of the 9-month follow-up period - see Figures 3.5 and 3.6 for patient flow through the study and description of withdrawals/dropouts. See Table 3.1 for a description of baseline characteristics of all patients recruited and enrolled to the study, and Table 3.2 for a summary of baseline characteristics of patients who dropped out throughout the duration of the study. Other data relating to the feasibility and patient acceptability of this intervention is discussed in greater detail in Chapter 4.

3.5.1.1. Adherence to the intervention and fidelity of intervention delivery

No formal data was obtained in relation to patients' adherence to the intervention throughout the VLCD phase. Similarly, no formal data was reported on the fidelity of the intervention delivery, although the intervention was delivered by largely the same two members of research team with each study visit following the same structure.

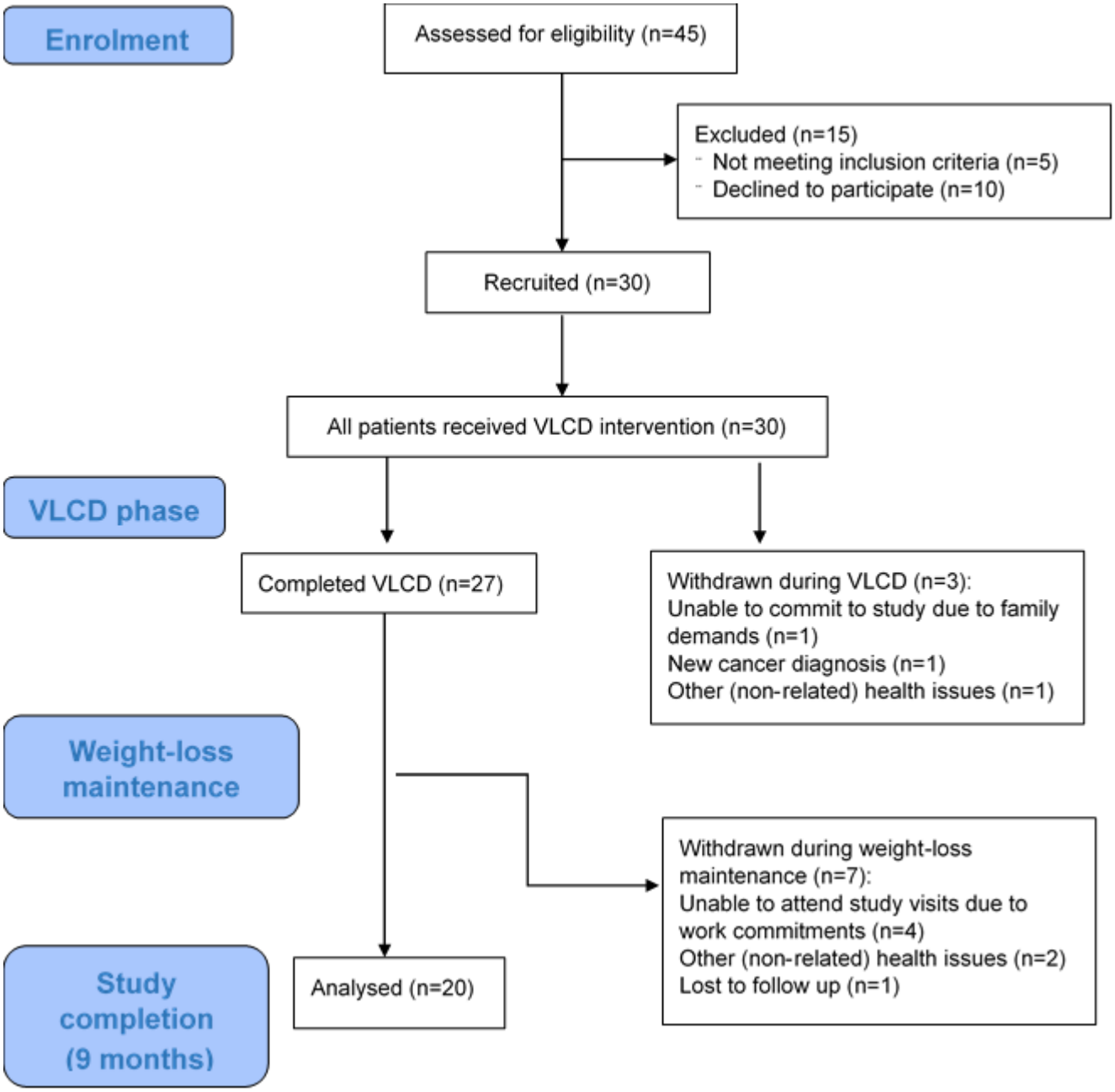


Figure 3.5. Patient flow throughout the study

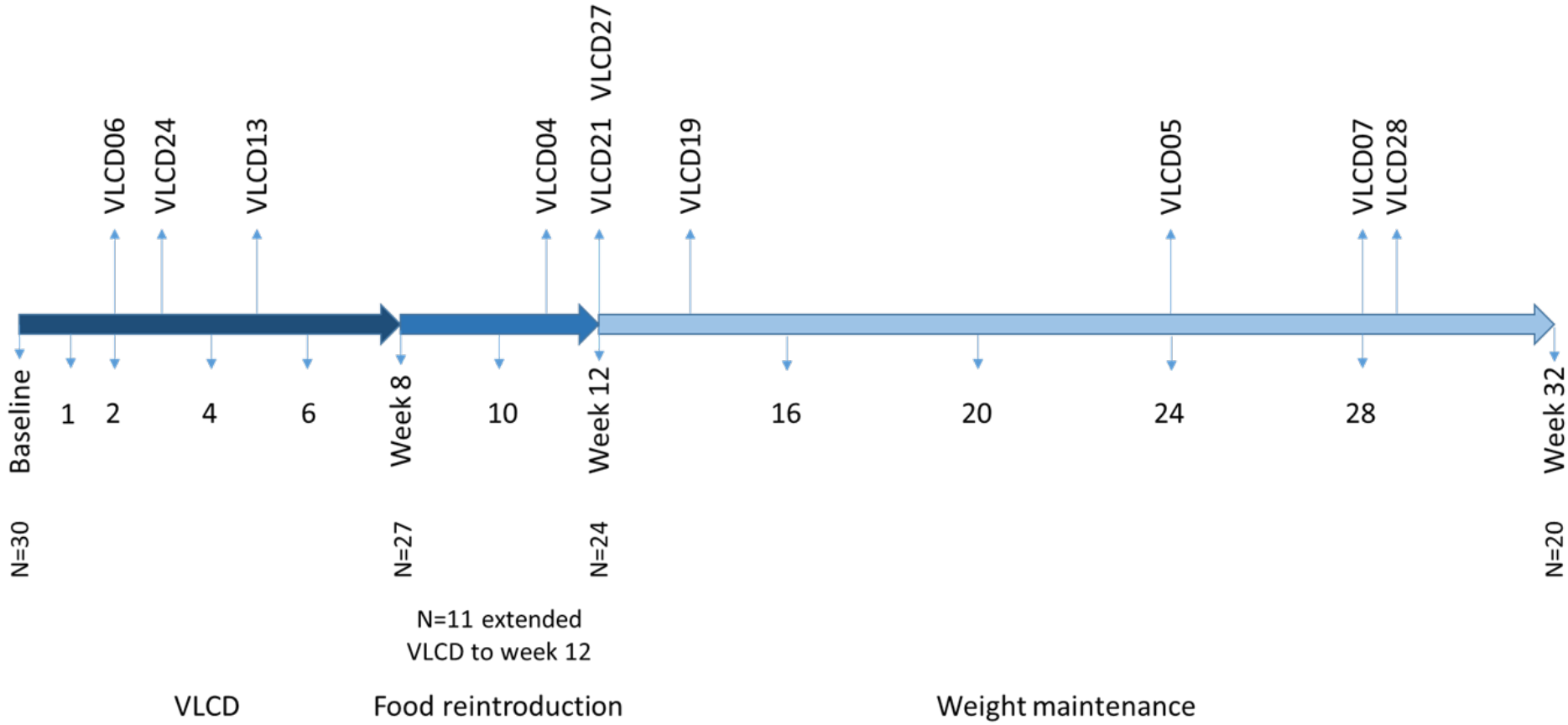


Figure 3.6. Timeline depicting withdrawal of patients from the study

Table 3.1. Baseline characteristics of patients recruited to the study

Subject Characteristics	**Baseline (n=30)**
Age (years)	56 ± 12
Sex (n) male/female	18/12
Time since NAFLD Diagnosis (months): Mean Median (range)	 28.4 ± 31.7 13.5 (1-113)
Anthropometry	
Weight (kg)	119 ± 25
Height (m)	1.7 ± 0.9
BMI (kg/m^2)	42 ± 8
Waist circumference (cm)	126 ± 16
Hip circumference (cm)[#]	126 ± 15
Fat mass (%)	45 ± 7
Skeletal muscle mass (kg):	29 ± 5
Blood pressure: systolic (mmHg) diastolic (mmHg)	144 ± 15 86 ± 11
Blood samples	
Total cholesterol (mmol/L)	4.3 ± 0.9
Triglycerides (mmol/L)	2.1 ± 1.8
HDL (mmol/L)	1.2 ± 0.3
LDL (mmol/L)	2.2 ± 0.8
AST (IU/L)	35 ± 18
ALT (IU/L)	47 ± 30
GGT (IU/L)	82 ± 74
Fasting glucose (mmol/L)	7.5 ± 2.3
HbA1c (mmol/mol)	50 ± 13
Insulin (pmol/L)	150 ± 104
Fibroscan (n=27)	

Stiffness (kPa) IQR (kPa)	13.0 ± 6.6 3.5 ± 3.0
Non-invasive scores	
FIB-4 NAFLD Fibrosis Score QRISK2 HOMA-IR	1.5 ± 1.0 -0.5 ± 1.9 15.5 ± 14.2 2.9 ± 1.8
Weight-related Quality of Life (OWLQOL) (n=27)	
Quality of Life Weight-related symptom measure	44 ± 26 46 ± 31

Table 3.2. Baseline characteristics of patients who withdrew from the study at post-VLCD (n=3) and follow-up (n=10)

Subject Characteristics	**Post-VLCD (n=3)**	**Follow-up (n=10)**
Age (years)	63 ± 18	51 ± 10
Sex (n) male/female	2/1	8/2
Time since NAFLD Diagnosis (months): Mean Median (range)	 30.5 ± 14.8 30.5 (20-41)	 38.5 ± 13.6 33.5 (4-96)
Anthropometry		
Weight (kg)	139.7 ± 43.5	128.1 ± 25.4
Height (m)	1.7 ±0.1	1.7 ± 0.1
BMI (kg/m^2)	47.8 ± 10.3	42.9 ± 9.3
Waist circumference (cm)	137.7 ± 14	131.8 ± 18.5
Hip circumference (cm)[#]	139.3 ± 9.7	125.7 ± 13.6
Fat mass (%)	48.2 ± 8.2	42.3 ± 3.0
Skeletal muscle mass (kg):	35.6 ± 12.5	35.4 ± 6.6
Blood pressure: systolic (mmHg) diastolic (mmHg)	139.3 ± 1.2 87.0 ± 11.4	140.5 ± 12.3 90.8 ± 9.8
Blood samples		

Total cholesterol (mmol/L)	3.6 ± 0.2	4.2 ± 0.3
Triglycerides (mmol/L)	1.3 ± 0.1	2.1 ± 0.9
HDL (mmol/L)	1.4 ± 0.4	1.0 ± 0.1
LDL (mmol/L)	1.7 ± 0.2	2.2 ± 0.7
AST (IU/L)	30.5 ± 14.9	26.7 ± 12.9
ALT (IU/L)	21.5 ± 6.4	39.8 ± 24.0
GGT (IU/L)	58.0 ± 15.6	46.8 ± 25.0
Fasting glucose (mmol/L)	6.9 ± 2.3	6.7 ± 1.6
HbA1c (mmol/mol)	43.5 ± 20.5	47.3 ± 11.0
Insulin (pmol/L)	155.5 ± 121.3	138.4 ± 119.0
Fibroscan		
Stiffness (kPa)	N/A	10.4 ±2.3
IQR (kPa)	N/A	1.8 ± 0.8
Non-invasive scores		
FIB-4	2.7 ± 2.2	0.9 ± 0.3
NAFLD Fibrosis Score	1.8 ± 0.4	0.6 ± 3.0
QRISK2	19.5 ± 16.4	9.8 ± 6.8
HOMA-IR	3.4 ± 2.1	2.2 ± 0.9
Weight-related Quality of Life (OWLQOL)		
Quality of Life	N/A	41.8 ± 26.0
Weight-related symptom measure	N/A	53.2 ± 31.9

N/A depicts where insufficient data had been obtained to calculate a mean ± SD. Data presented at follow includes the data (n=-3) of those dropped out during the VLCD.

3.5.1.2. Irrespective of completion (IOC) analysis of weight change at 9-months

Overall 34% (n=10) of patients achieved the primary outcome of a sustained ≥10% weight loss at 9-months follow-up, 51% achieved ≥7% weight loss and 68% achieved ≥5% weight loss. Mean weight loss was 10.3 ± 10.3 kg (range: -42.2 to +6.8 kg) or 8.9 ± 8.1% (range: -29.5 to +5.2%). At 9-months, those who completed 12-weeks of the VLCD had maintained significantly more weight loss than those who completed 8-weeks of the VLCD (13.4 ± 7.8% vs 4.4 ± 5.4%, p=0.002).

3.5.1.3. IOC analysis of weight change post-VLCD phase

At the end of the VLCD phase, 53% (n=16) of patients achieved ≥10% weight loss, 63% achieved ≥7% weight loss and 77% achieved ≥5% weight loss. Mean weight loss was 11.3 ± 7.7 kg (range: -38.7 to +1.7 kg) or 9.7 ± 5.8% (range: -26.4 to +1.3%). Post VLCD, those who completed 12-weeks of the VLCD had lost significantly more weight than those who completed 8-weeks (13.6 ± 5.1% vs 7.2 ± 4.6%, p=0.002).

3.5.1.4. Adverse events

No treatment related serious adverse events were reported during the study. The most common side effects reported during the VLCD phase were constipation, dizziness, headaches, and increased sensitivity to cold, reported by 37%, 19%, 11% and 7% of patients respectively. No side effects were reported during food reintroduction and follow-up.

3.5.1.5. Baseline characteristics

60% of patients recruited were male and mean age was 56 ± 12 years. The mean weight and BMI at baseline were 119 ± 25 kg and 42 ± 8 kg/m^2 respectively. At baseline, 14 (47%) patients had a BMI between 30-40 kg/m^2, 13 (43%) had a BMI between 40-50 kg/m^2 and 3 (10%) had a BMI >50 kg/m^2 (see Tables 3.1 and 3.5). Overall, 16 (53%) patients had T2DM and 13 (43%) patients had the full metabolic syndrome, at baseline as defined by Huang et al. (Huang, 2009, Zimmet et al., 2005).

All patients had either an intermediate/high NFS or intermediate/high FIB-4; 16/30 also had NASH with fibrosis on biopsy (2 with F1, 6 with F2, 5 with F3 and 3 with F4), as reported using the Kleiner (Kleiner et al., 2005) scoring system. The baseline LSM was 13.0 kPa (± 6.0 kPa; n=27) and 17 had an LSM >8 kPa. Baseline NFS and FIB4 were -0.05 (± 2.1) and 1.5 (± 1.0) respectively (Table 3.1).

3.5.1.6. Per-protocol analysis of weight and body composition outcomes

All patients completing the VLCD (n=27) lost weight and maintained weight loss at 9-months follow-up. 59% (n=16) of those who completed the VLCD phase achieved ≥10% weight loss post-VLCD. Mean weight loss immediately after the VLCD, in those completing the intervention, was 12.6 ± 7.7 kg (range: -38.7 to -3.2 kg) or 10.8 ± 5.8% (range: -26.4 to -3.3%), as shown in Figure 3.7. Weight loss at 12-weeks for all patients completing the VLCD (regardless of length of VLCD) was 12.9 ± 8.3kg and 11.4 ± 6.1%. Overall, 80%, 75%, and 50%

of patients achieved ≥5%, ≥7% and ≥10% weight loss respectively at 9-month follow-up, and the mean overall weight loss was 13 kg (range: -42.6 to -0.3 kg) (12% of total body weight).

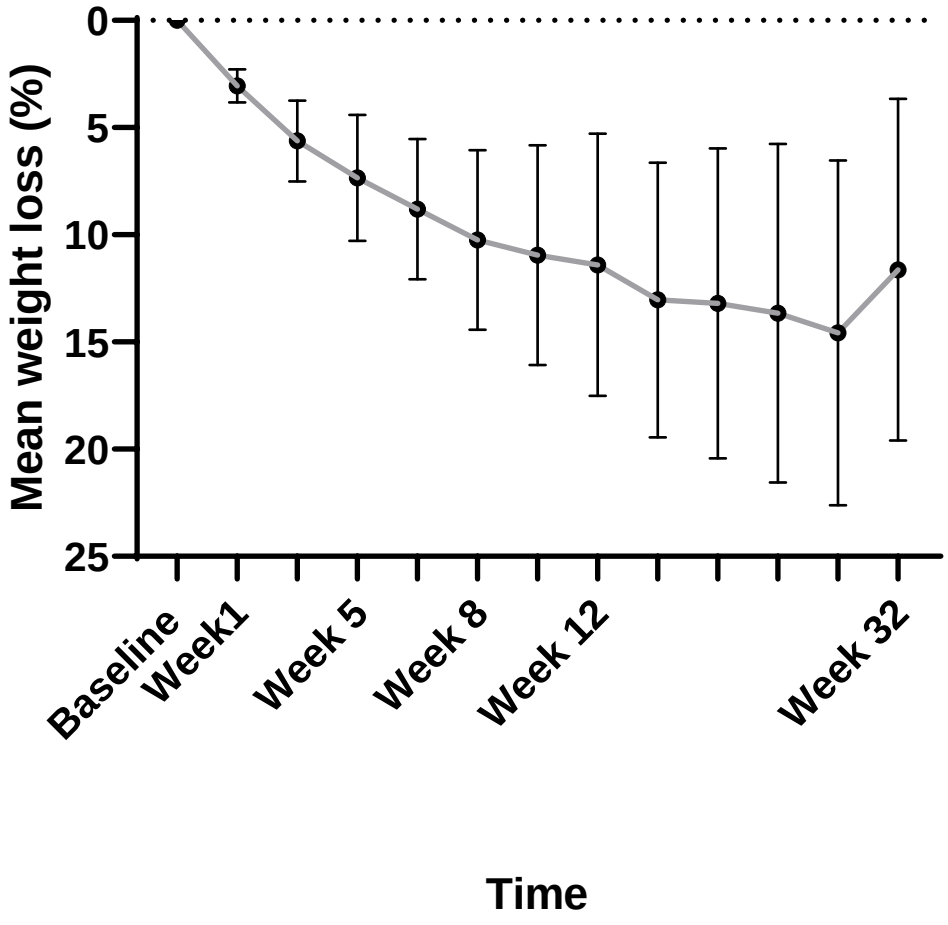


Figure 3.7. *Percentage weight loss for the duration of the study. 16 patients completed the VLCD phase at week 8 (visit 6), while 11 patients extended the VLCD phase to week 12 (visit 8). 20 patients completed the 9-month visit (visit 13).*

Between the end of the VLCD and 9-month follow-up, 45% of patients lost further weight (mean further weight loss of 3.3 kg (range: -11.0 to -0.8 kg)) and 55% regained weight, with a mean overall weight regain of 3.2kg (range: 1.3 to 4.8 kg) from their post-VLCD weight, equivalent to 3.4% (range: 0.9 to 5.7%). Following weight regain, no patients exceeded their baseline weight at 9-months. Mean BMI decreased from 40 kg/m^2 (range: 30.3 to 62.3 kg/m^2) at baseline to 35 kg/m^2 (range: 26.3 to 58.8 kg/m^2) post-VLCD and 35kg/m^2 (range: 27.5 to 57.8 kg/m^2) at 9-month follow-up. Moreover, mean total body fat mirrored these findings falling from 45% to 41% post-VLCD and 41% at 9-months. Skeletal muscle mass did not change

significantly between baseline and post-VLCD (29 ± 5 kg vs. 27 ± 5 kg, p=0.219), or between post-VLCD and 9-month follow-up (27 ± 5 kg vs. 26 ± 6 kg, p=0.617). However, there was a significant decrease observed between baseline and 9-months (29 ± 5 kg vs. 26 ± 6 kg, p=0.009).

3.5.2. Secondary outcomes

3.5.2.1. Liver health

Figure 3.8 presents the changes in ALT, AST and GGT throughout the VLCD intervention and through the maintenance period to 9-month follow-up. Overall, liver enzymes significantly improved from baseline to post-VLCD, and these improvements were maintained at 9-months. Interestingly, there was a significant rise in liver enzymes one week into the VLCD that had returned to baseline by week four. There were no significant relationships between total weight loss (%) and change in AST (r=0.365, p=0.061), ALT (r=0.215, p=0.281) or GGT (r=0.181, p=0.377) over the study period in the whole cohort or in the subset of patients with elevated liver enzymes at baseline. There were no significant changes in bilirubin or platelets throughout the study period (p>0.05).

Figure 3.9 indicates the changes throughout the VLCD intervention in AST and ALT in a subsection of patients (n=15) who presented with abnormally elevated enzymes at baseline (AST>35 IU/L and ALT≥45 IU/L). This depicts a significant reduction in ALT and AST between baseline and post-VLCD, with an initial increase observed within the first week (between baseline and visit 2), with a subsequent reduction to approximately baseline values over the following two weeks (between visit 2 and visit 3). This is largely similar to the pattern of change shown in ALT and AST for the whole cohort.

LSM (Figure 3.8) also improved significantly between baseline and post VLCD (12.7 ± 7.4 kPa to 7.5 ± 2.3 kPa) and this was maintained at 9-month follow-up (6.9 ± 2.0 kPa, p=0.001).

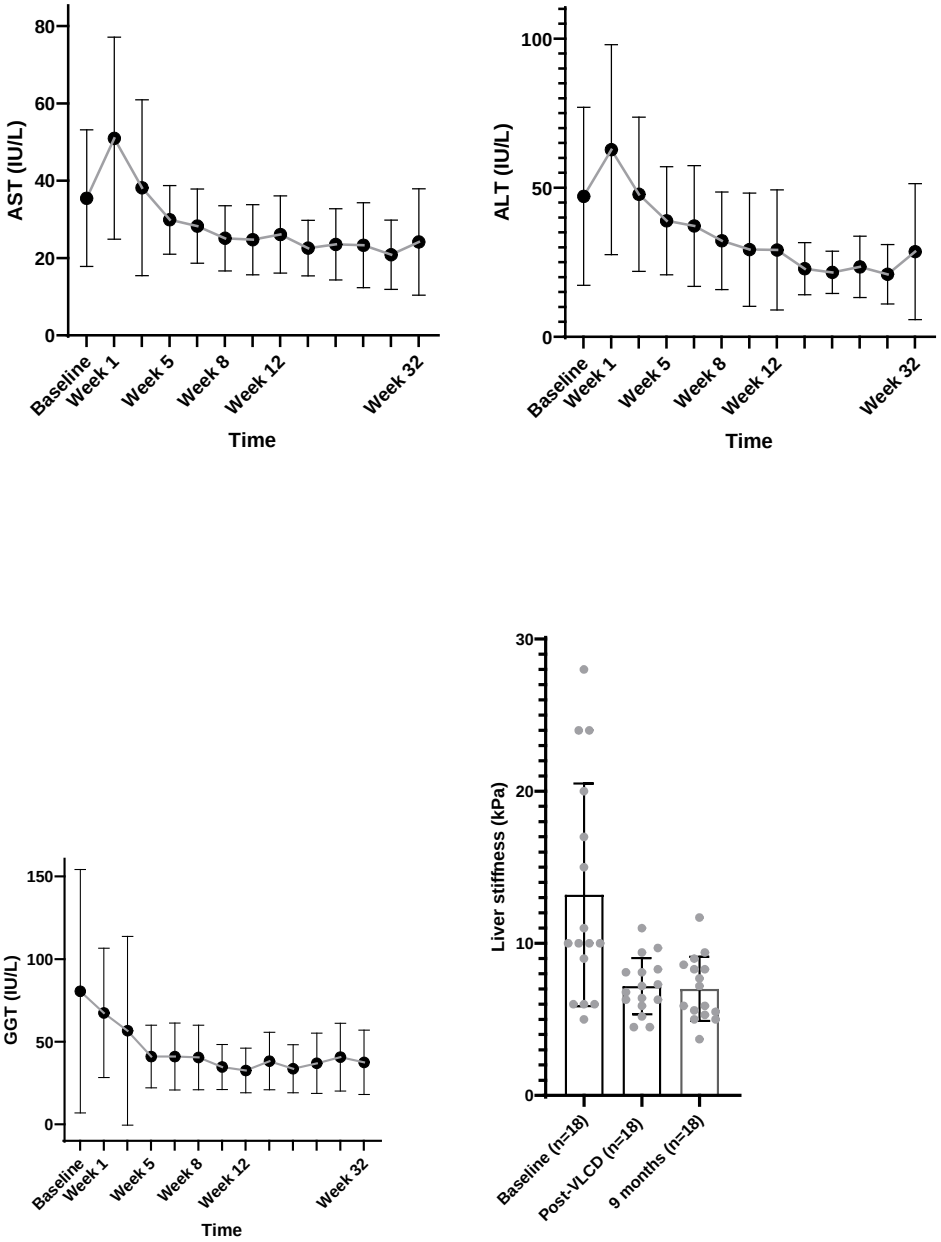


Figure 3.8. *Liver health: AST, ALT and GGT for the duration of the study (n=30 at baseline, n=28 at visit 3, n=27 at visit 5, n=20 at visit 13). Liver stiffness (kPa) at baseline, post-VLCD and 9-months.*

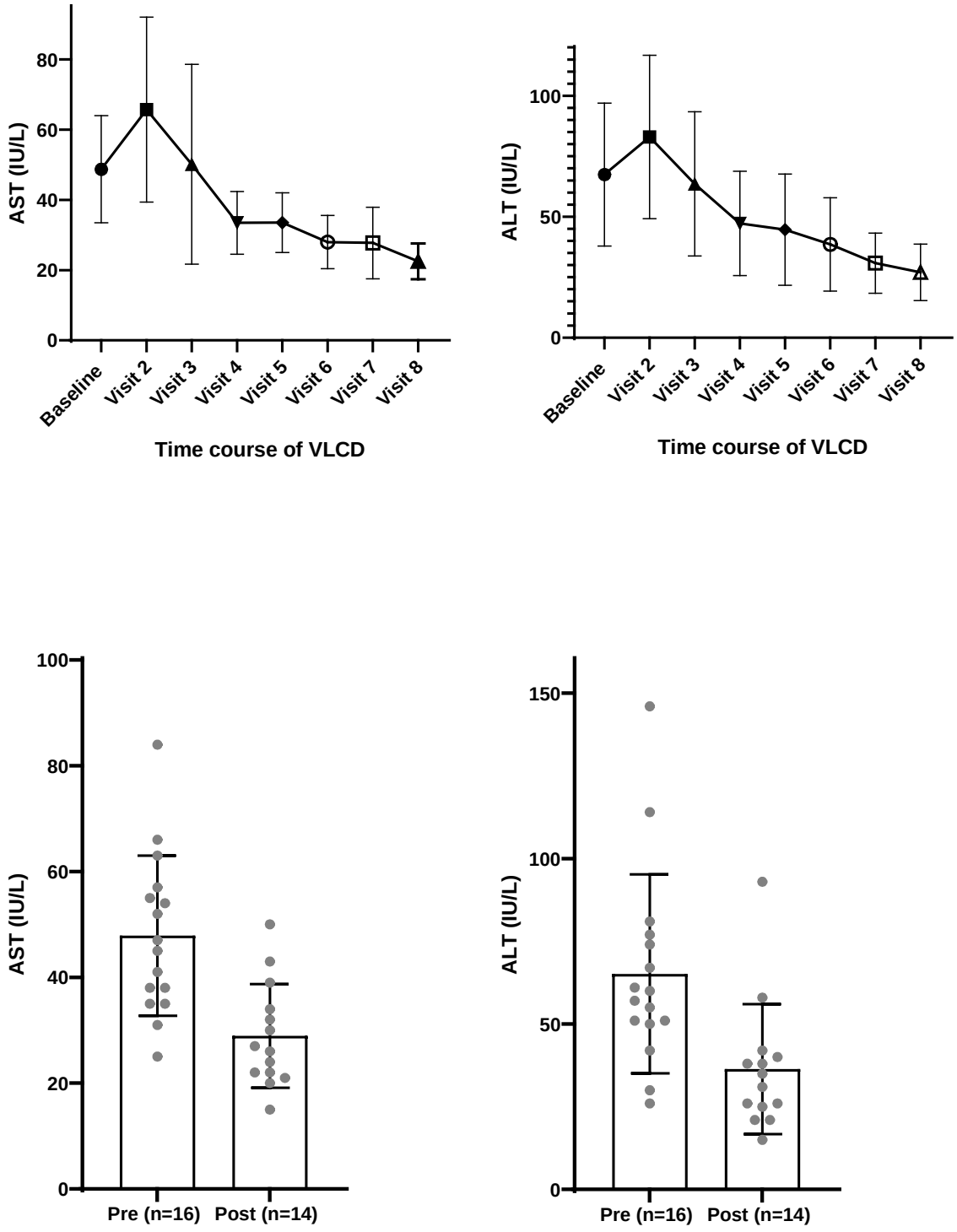


Figure 3.9. Changes in ALT and AST throughout VLCD intervention in those with elevated AST and ALT values. Between baseline and visit 6, n=27. Between visit 6 and visit 8, n=11, reflecting the 11 participants who elected to extend the VLCD phase.

3.5.2.2. *Metabolic control*

Metabolic control (Glucose, HbA1c and insulin; Figure 3.5 and Table 3.5) improved from baseline to post-VLCD and these improvements were maintained at 9-months. Overall, 47% of patients were prescribed oral antidiabetic medications at baseline and this reduced to 30% at 9-month follow-up. Three patients (10%) had their diabetes medications withdrawn altogether and five other patients (16%) had their dosage reduced. At 9-months, 9/12 patients with diabetes had achieved good control of their diabetes (HbA1c <48mmol/mol) (Oze-Fukai et al., 2008). Insulin sensitivity also improved with a reduction in HOMA-IR from 3.2 at baseline to 2.1 post-VLCD, to 1.8 at 9-month follow-up.

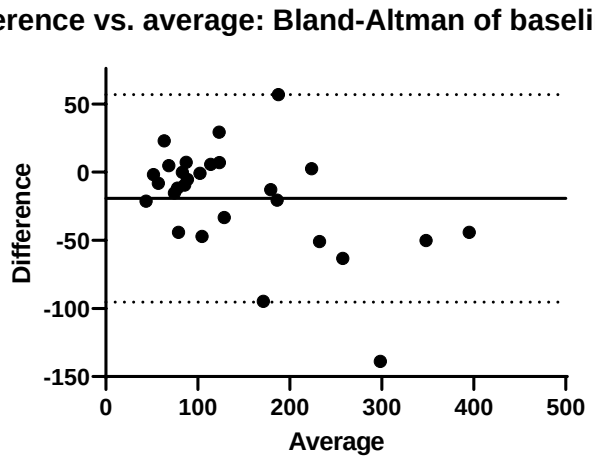
3.5.2.2.1. Discrepancies between insulin analyses

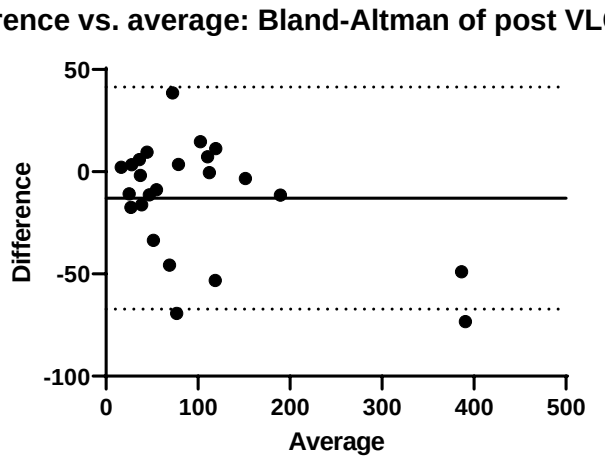
Initial analysis of insulin data was determined to not be adequate due to large amounts of missing or anomalous data. Therefore, repeated analysis was undertaken, which elicited significantly different results to initial results. Initial analyses provided sample numbers of 28, 25 and 14 at baseline, post-VLCD and at follow up, respectively. Repeat analysis provided sample numbers of 29, 27 and 20 at baseline, post-VLCD and at follow up, respectively. Data reported from the secondary analyses of insulin had a small coefficient of variance (CV), whereas this data was not reported with the primary analyses. Furthermore, a much greater sample size was successfully analysed in the secondary analysis and the reported changes in insulin were consistent with NAFLD improvement.

Table 3.3. Differing means ± SD between the first and second analyses of insulin at baseline, post-VLCD and follow up.

	1st analysis	2nd analysis
Baseline	135 ± 85	150 ± 104
Post-VLCD	92 ± 91	118 ± 133
Follow up	138 ± 77	92 ± 72

Figure 3.10 shows the variance between the insulin analyses. Insulin, and insulin related data discussed going forward is presented using the secondary analyses that was completed. This is due to the significantly larger sample sizes within this analysis, and significantly fewer anomalous results.





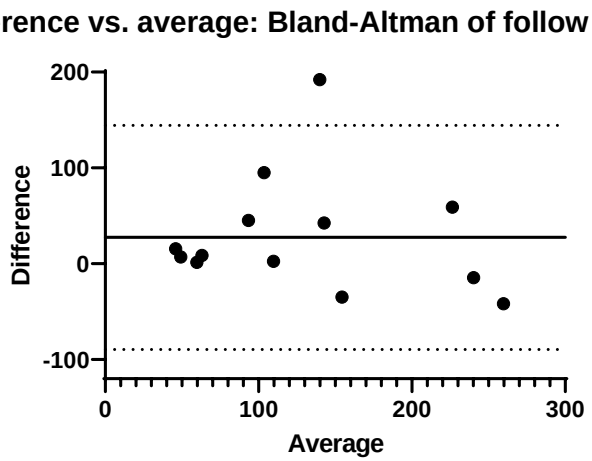


Figure 3.10. Bland-Altman plots depicting the differences in results obtain between insulin assays undertaken by NuTH hospitals (1st analysis) and by ourselves within the Newcastle University laboratories (2nd analysis) at baseline, post-VLCD and follow-up.

3.5.2.3. Cardiovascular disease (CVD) risk

Cardiovascular changes seen during the study period are shown in Figure 3.5 and Table 3.5. Overall, there was a significant reduction in blood pressure from 148/85 mmHg to 134/81 mmHg post-VLCD, which elevated slightly at 9-month follow-up, but did not exceed baseline with a mean blood pressure of 138/83 mmHg. QRISK2, a measure of 10-year risk of cardiovascular events, reduced significantly from 17.1% to 12.6% post-VLCD suggesting a global improvement in CVD risk. This also increased slightly at 9-month follow-up but did not exceed baseline with a final QRISK2 score of 13.3%. QRISK2 fell from >10%, a treatment threshold determined by NICE for primary prevention of CVD, to <10% for 5 (19%) patients post-VLCD and 12 (60%) of those who completed the 9-month follow-up phase (NICE, 2016b, England, 2013).

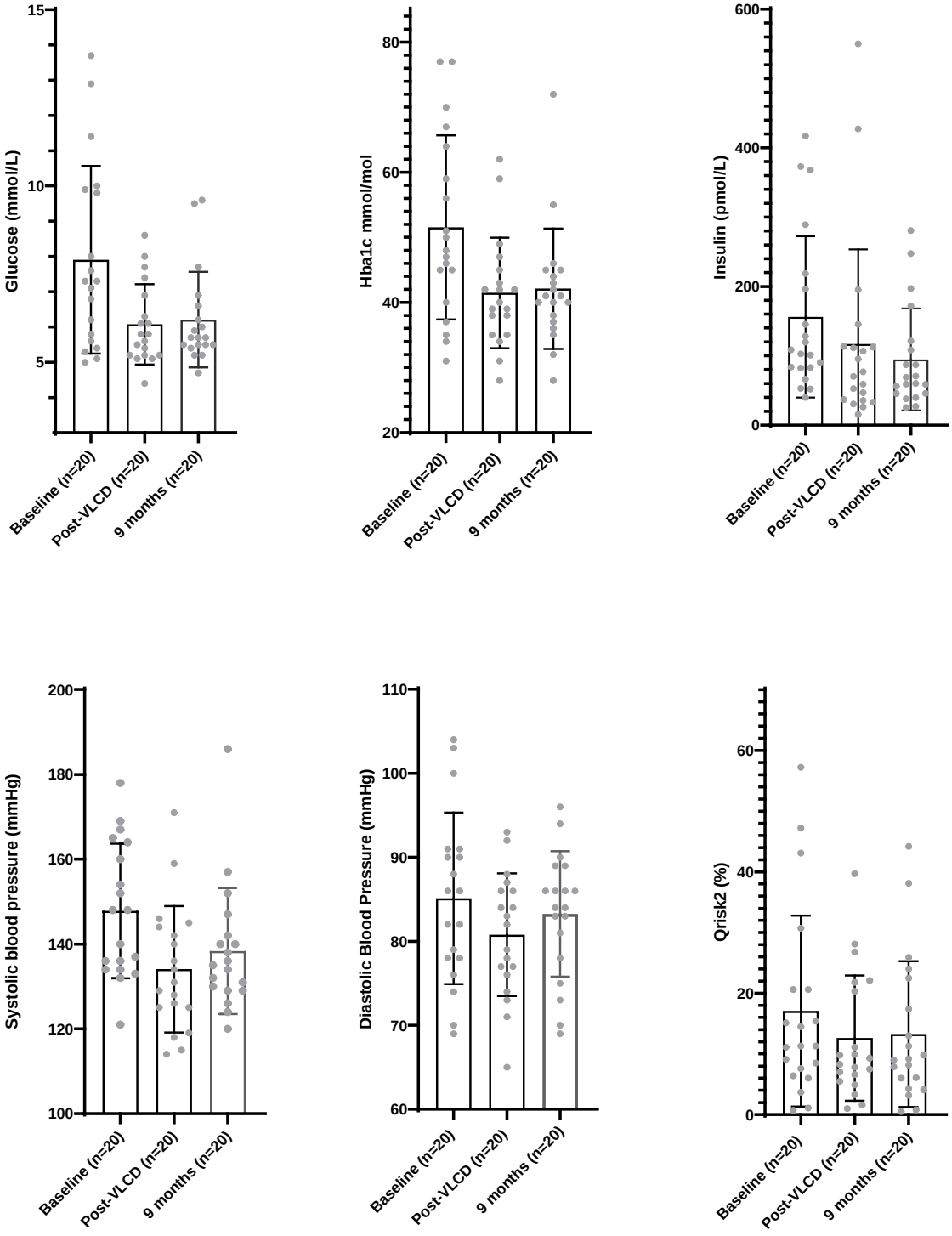


Figure 3.11. Cardiometabolic risk factor changes throughout study period. Analysis in patients with complete data sets.

3.5.2.4. Quality of life (QoL)

Patients reported a significantly increased quality of life at 9-month follow-up with a decrease in weight-related symptoms. QoL score improved from 42 at baseline to 57 post-VLCD, and 56 at 9-months follow-up (Figure 3.12). Weight-related symptoms score improved from 44 at baseline to 28 post-VLCD and 28 at 9-months follow-up.

In addition, 30% of patients reduced the number of medications they were taking during the study (see Table 3.4), with polypharmacy reducing from 7 patients at baseline to 5 patients post VLCD to patients at follow-up. Mean number of medications being taken decreased from 2.5 per patient at baseline to 2.3 patients post-VLCD to 2 patients at follow-up. Table 3.4 further depicts the changes in different types of medications; antihyptertensive, antidiabetic, statins and antidepressants.

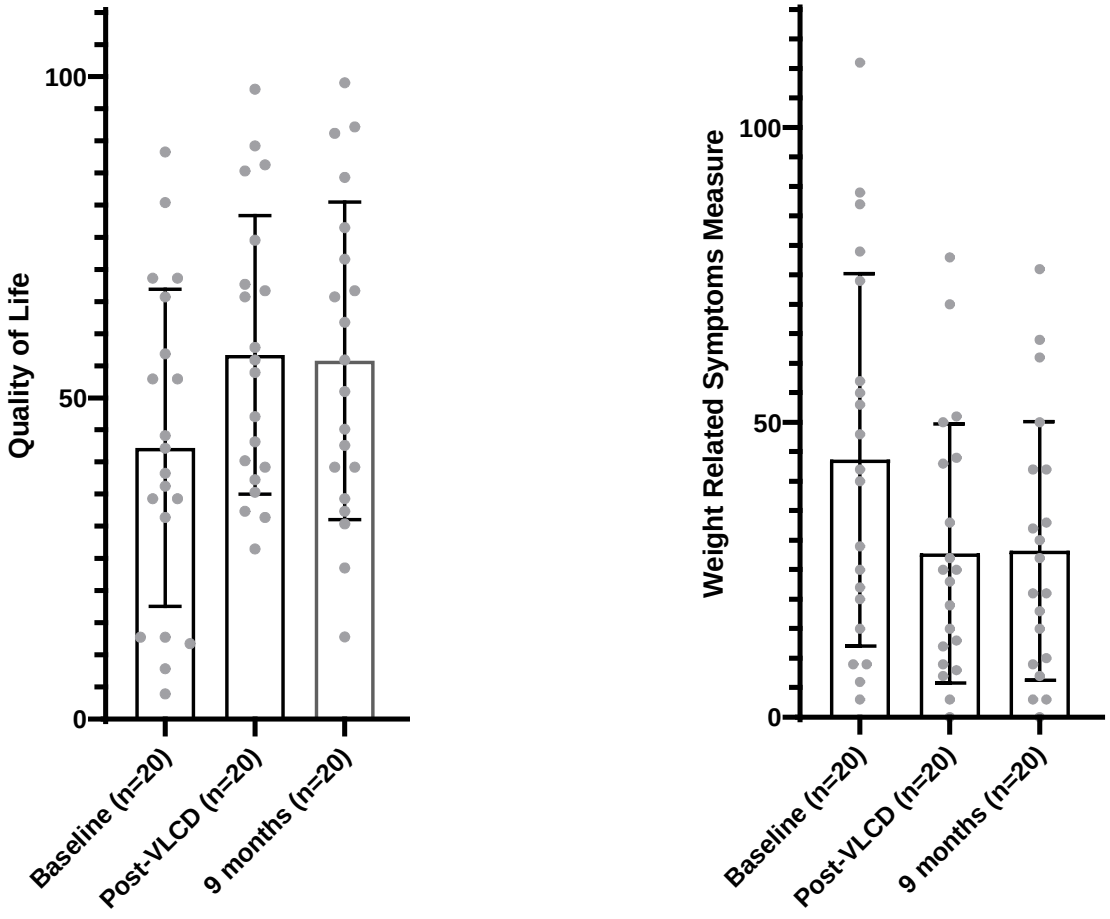


Figure 3.12. Quality of Life and Weight Related Symptoms at key time points in the study. (An increase in QoL scores indicates better QoL and a decrease in weight-related symptoms indicates an improvement). Analysis in patients with compete datasets.

Table 3.4. Changes in medications across the VLCD intervention

	Number of patients on medications at baseline (out of 30)	**Number of patients on medications at baseline (out of 27)**	**Number of patients on medications at 9-months (out of 20)**
Statins:	16 (53%)	16 (59%)	10 (50%)
Antihypertensive:	15 (50%)	14 (52%)	8 (40%)
Antidepressants:	6 (20%)	5 (19%)	3 (15%)
Antidiabetic:	14 (46%)	11 (41%)	7 (35%)
1 Antidiabetic drug	8	8	5
2 Antidiabetic drugs	3	3	2
3 Antidiabetic drugs	3	0	0
Total number of meds (mean):	2.5	2.3	2.0
>5	7	5	3

Table 3.5. Subject characteristics. Statistical analysis in patients with complete datasets.

Subject Characteristics	**Baseline (n=20)**	**Post-VLCD (n=20)**	**9-month (n=20)**	**Overall P-value**	**Baseline vs Post-VLCD p-value**	**Baseline vs 9-month p-value**
Age (years)	57 ± 11	55 ± 11	57 ± 11			
Sex (n) male/female	18/12	17/10	10/10			
Time since NAFLD Diagnosis (months): Mean Median (range)	 25.1 ± 32.9 10.5 (1-113)					
Anthropometry						
Weight (kg)	113 ± 20	99 ± 18	100 ± 18	<0.001***	<0.001***	<0.001***
Height (m)	1.7 ± 0.9					
BMI (kg/m^2)	40 ± 8	35 ± 7	35 ± 8	0.004**	<0.001***	<0.001***
Waist circumference (cm)	121 ± 14	107 ± 13	104 ± 13	<0.001***	<0.001***	<0.001***
Hip circumference (cm)[#]	122 ± 14	115 ± 15	114 ± 15	0.002**	0.023*	0.003**
Fat mass (%)	45 ± 7	41 ± 10	41 ± 10	0.039*	0.009**	0.004**
Skeletal muscle mass (kg):	29 ± 5	27 ± 5	26 ± 6	0.009**	0.219	0.009**

Blood pressure: systolic (mmHg) diastolic (mmHg)	148 ± 16 85 ± 10	134 ± 15 81 ± 7	138 ± 15 83 ± 7	0.009** 0.207	0.006**	0.360
Mean weight loss (%):		11 ± 6	12 ± 8	0.667		
Mean weight loss (%); IOC (n=30):		10 ± 6	9 ± 8	0.061		
Blood samples						
Total cholesterol (mmol/L)	4.4 ± 1.0	4.3 ± 1.2	4.3 ± 1.2	0.491		
Triglycerides (mmol/L)	2.3 ± 2.0	2.0 ± 1.5	2.0 ± 1.7	0.049*	0.079	0.113
HDL (mmol/L)	1.2 ± 0.3	1.2 ± 0.4	1.3 ± 0.4	0.251		
LDL (mmol/L)	2.3 ± 0.9	2.1 ± 0.9	2.2 ± 1.0	0.145		
AST (IU/L)	39 ± 19	26 ± 9	24 ± 14	<0.001***	0.009**	0.002**
ALT (IU/L)	52 ± 33	30 ± 17	29 ± 23	<0.001***	0.012*	0.002**
GGT (IU/L)	76 ± 43	37 ± 20	37 ± 20	<0.001***	<0.001***	<0.001***
Fasting glucose (mmol/L)	7.9 ± 2.7	6.1 ± 1.1	6.2 ± 1.4	0.046*	0.028*	0.047*
HbA1c (mmol/mol)	52 ± 14	41 ± 9	42 ± 9	<0.001***	<0.001***	0.002**
Insulin (pmol/L)	156 ± 116	117 ± 136	95 ± 74	0.002**	0.034*	0.005*
Fibroscan						
Stiffness (kPa)	12.7 ± 7.4	7.5 ± 2.3	6.9 ± 2.0	<0.001***	0.009**	0.004**
IQR (kPa)	4.0 ± 4.3	2.4 ± 3.0	1.7 ± 1.1	0.107		
Non-invasive scores						
FIB-4	1.5 ± 0.8	1.3 ± 0.6	1.2 ± 0.5	0.082		

NAFLD Fibrosis Score	-0.5 ± 1.9	-0.8 ± 1.4	-0.9 ± 1.4	0.163		
QRISK2	17.1 ± 15.7	12.6 ± 10.3	13.3 ± 12	0.027*	0.074	0.085
HOMA-IR	3.2 ± 2.3	2.1 ± 2.1	1.8 ± 1.4	0.002**	0.212	0.005**
Weight-related Quality of Life (OWLQOL)						
Quality of Life	42 ± 25	57 ± 22	56 ± 25	0.005*	<0.001***	0.049*
Weight-related symptom measure	44 ± 32	28 ± 22	28 ± 22	0.005*	0.024*	0.021*

*denotes significance of p<0.05

**denotes significance of p<0.01

***denotes significance of p<0.001

3.5.3. Analyses of drop outs

Three patients withdrew from the study during the VLCD phase and ten by 9-month follow-up. Figures 3.2 and 3.3 depict the main reasons for, and a timeline of when patients withdrew, from baseline until follow-up. Table 3.6 describes the characteristics of those who had dropped out by both major time points. Weight and total weight loss were obtained from the most recent visit prior to withdrawal. When compared to the post-VLCD and follow-up data to those who completed the intervention (see section 3.5.2, Table 3.5), there is a significant difference in weight, and total weight loss. This is perhaps due to patients not receiving the full benefit of the intervention, in the case of those who had withdrawn throughout the VLCD phase. However, those who withdrew post-VLCD, during the weight maintenance/ follow-up period, had received the full benefit of the intervention. Given that the total weight loss (kg/%) was lower than that those who remained engaged for the full duration of the intervention, it is possible that those who withdrew did so because they had achieved a lesser weight loss than perhaps anticipated.

Table 3.6. Demographics and other characteristics of those patients who withdrew prematurely from the intervention, compared to the baseline cohort (n=30).

Characteristic	**Post-VLCD (n=3)**	**Follow-up (n=10)**	**Baseline cohort (n=30)**
Gender (m/f)	2/1	8/2	18/12
Age (years)	63 ± 3	55 ± 13	56 ± 12
Deprivation score	27 ± 22	30 ± 19	29 ± 19
Deprivation quintile	3.5 ± 1.5	3.4 ± 1.5	3.5 ± 1.5
Weight (kg)	153 ± 55	126 ± 31	119 ± 25
Total weight loss (kg)	1.6 ± 4.5	3.5 ± 5.8	
Total weight loss (%)	1.7 ± 3.5	2.9 ± 4.4	

There were no significant differences observed in mean deprivation scores (p=0.775) or mean deprivation quintile (p=0.734) between those who withdrew and those who completed the study at follow-up. Similarly, age did not significantly vary between those who withdrew and those who continued to engage with the intervention up to follow-up (p=0.677). A significantly greater proportion of males withdrew from the intervention than females. At baseline, there were 18 males and 12 females recruited, and by 9-month follow-up, there were 10 males and 10 females. The cohort who remained engaged were 50% male, 50% female, compared to those who withdrew; 80% male and 20% female.

Total weight loss (kg) was significantly different between those who withdrew and those who completed the study at follow-up (p=0.003). Similarly, total weight loss (%) was significantly different between those who withdrew and those who completed the study at follow-up (p=0.001).

When compared to the baseline cohort, it can be seen that there are no defining variables that may indicate a higher chance of dropping out. For example, age is similar, as is deprivation. However, given the small numbers of those who did drop out, it is challenging to make sophisticated comparisons between baseline and drop outs.

3.5.4. The effect of losing </>10% body weight: an exploratory analyses

The primary outcomes were acceptability and feasibility of recruiting to and delivering the intervention, and to determine the percentage of patients achieving 10% weight loss. Post-VLCD, 11 (41%) of patients had lost <10% of their initial body weight and 16 (59%) had lost >10% (n=27). At follow-up, 10 (50%) of patients had lost <10% of their initial body weight and 10 (50%) had lost >10% (n=20).

3.5.4.1. Side effects

Throughout the VLCD intervention, minor side effects were reported, as described in section 3.5.1.3. In patients that lost >10% at the end of the VLCD intervention, side effects were less common, with fewer patients experiencing headaches, dizziness, and increased sensitivity to cold, but more experiencing constipation, as indicated in Table 3.7.

Table 3.7. Prevalence of side effects in patients who lost </>10% weight loss during the VLCD intervention

side effects	**<10% weight loss**	**>10% weight loss**
Constipation	2/11 (18%)	8/16 (50%)
Increased sensitivity to cold	2/16 (13%)	0 (0%)
Headaches	2/16 (13%)	1/11 (9%)
Dizziness	4/16 (25%)	1/11 (9%)

3.5.4.2. Baseline characteristics

Observed differences in baseline characteristics between patients who achieved </>10% weight loss at follow-up are indicated in Table 3.8. There were no significant differences observed in any of the baseline characteristics, with the exception of LDL (p=0.006).

Table 3.8. Mean baseline characteristics of patients who lost </>10% weight at follow-up

Subject characteristics	**<10% weight loss (n=10)**	**>10% weight loss (n=10)**	**P value**
Age (years)	55±12	58±11	0.596
Sex (m/f)	4/6	6/4	
Deprivation Score: Quintile:	 31±16 4±1	 26±23 3±2	 0.589 0.140
Time since NAFLD Diagnosis (months):	17±26	33±38	0.273
Anthropometry			
Weight (kg)	111±23	115±17	0.614
BMI (kg/m^2)	40±10	41±5	0.757
Waist circumference (cm)	118±15	126±12	0.206
Hip circumference (cm)	125±19	123±14	0.793

Fat mass (%)	45±10	45±5	0.933
Skeletal muscle mass (%)	29±6	31±6	0.587
Blood pressure:			
systolic (mmHg)	146±19	148±19	0.737
diastolic (mmHg)	88±9	82±10	0.154
Blood samples			
Total cholesterol (mmol/L)	5±1	4±1	0.088
Triglycerides (mmol/L)	1.9±0.8	2.8±2.7	0.372
HDL (mmol/L)	1.3±0.4	1.1±0.3	0.403
LDL (mmol/L)	2.7±0.8	1.7±0.5	0.006**
AST (IU/L)	39±21	40±17	0.973
ALT (IU/L)	60±43	45±19	0.337
GGT (IU/L)	81±51	71±34	0.641
Fasting glucose (mmol/L)	7±3	9±3	0.213
HbA1c (mmol/mol)	50±14	54±14	0.557
Insulin (pmol/L)	132±87	151±101	0.681
Fibroscan			
Stiffness (kpa)	11±6	15±8	0.332
Non-invasive scores			
FIB-4	1.3±0.7	1.7±0.8	0.275
NAFLD Fibrosis Score	-1.1±1.9	-0.04±1.7	0.234
QRISK2 (%)	12±12	22±18	0.176
HOMA-IR	2.6±1.6	3.1±2.0	0.592
Weight related quality of life			
Quality of Life	48±25	37±25	0.338
Weight-related symptom measure	32±24	56±35	0.096

*denotes significance of <0.05

**denotes significance of <0.01

***denotes significance of <0.001

3.5.4.3. *Early weight loss as a predictor of achieving >10% weight loss*

Those that took part in a semi-structured one to one interview immediately following the VLCD intervention, reported that early weight loss acted as a motivator to adhere to the intervention (see Chapter 4 for full qualitative analysis). Weight loss (% of starting body weight) achieved at three weeks into the intervention was found to be significantly correlated with total weight loss at follow-up under both per-protocol analyses (r=0.636, p=0.003) and intention-to-treat analyses (r=0.446, p=0.015), shown in Figure 3.13.

Figure 3.13. Correlations between early weight loss (weight loss at 3 weeks) and final weight loss. A) indicates the 'full dataset' analyses and B) indicates the irrespective of completion analyses

3.5.4.4. *Liver health*

At follow-up, patients who had lost less than 10% weight loss did not experience significantly different measures of liver health, with the exception of GGT. Patients who lost greater than 10% weight loss had significantly lower GGT that those who did not at follow-up (27 vs. 37 IU/L, p=0.018), as summarised in Table 3.9.

While no other indicators of liver health were significantly different between groups who lost </>10% weight loss, AST and ALT are approaching significance (p=0.058 and p=0.07 respectively). While not statistically significant, these results are likely to be clinically significant.

Table 3.9. Measures of liver health in patients who had lost </>10% weight loss at follow-up.

Variables	**<10% weight loss (n=10)**	**>10% weight loss (n=10)**	**P value**
AST (IU/L)	30±16	18±9	0.058
ALT (IU/L)	38±29	19±6	0.070
GGT (IU/L)	47±22	27±10	0.018*
Liver stiffness (kpa)	7±2	7±2	0.466
FIB-4 score	1.2±0.6	1.2±0.5	0.937
NFS	-0.9±1.6	-1.0±1.3	0.853

*denotes statistical significance

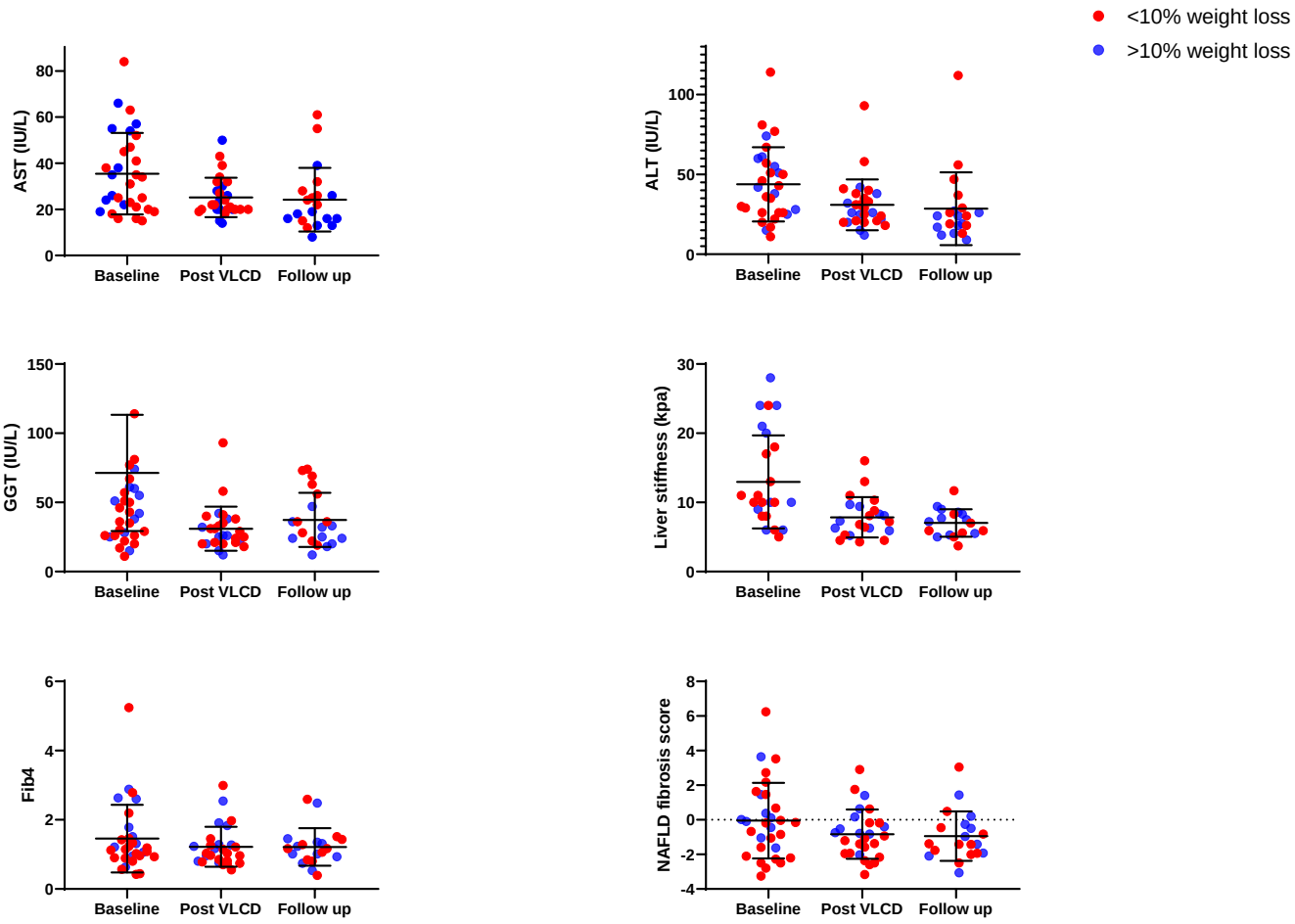


Figure 3.14. The changes in indicators of liver health at baseline, post-VLCD and follow-up in patients who lost </>10% weight loss. This Figure indicates the changes of A) AST (IU/L), B) ALT (IU/L), C) GGT (IU/L), D) liver stiffness (kpa), E) FIB-4 score and F) NFS.

3.5.4.5. *Metabolic control*

At follow-up, patients who had lost less than 10% weight loss did not experience significantly different measures of metabolic control, shown in Table 3.10 and Figure 3.14.

Table 3.10. Measures of metabolic control in patients who lost </>10% weight loss at follow-up.

Variables	**<10% weight loss (n=10)**	**>10% weight loss (n=10)**	**P value**
Fasting glucose (mmol/L)	5.8±0.5	6.6±1.7	0.164
HbA1c (mmol/mol)	40±4	44±12	0.474
Insulin (pmol/L)	133±80	144±81	0.812
HOMA-IR	2.2±1.3	2.8±1.6	0.466
HOMA-B	119±62	104±50	0.651
HOMA-S	60±29	51±36	0.663

3.5.4.6. *Cardiovascular disease risk*

At follow-up, patients who had lost less than 10% weight loss showed significantly higher levels of total cholesterol (4.8 vs. 3.8 mmol/L, p=0.035) and LDL (3.1 vs. 1.5 mmol/L, p<0.001) than those who lost greater than 10% of total body weight, shown in Table 3.11 and Figure 3.15.

Table 3.11. Measures of cardiovascular disease risk in patients who lost </>10% weight loss at follow-up.

Variables	**<10% weight loss (n=10)**	**>10% weight loss (n=10)**	**P value**
Fat mass (%)	44±11	38±8	0.242
Skeletal muscle mass (kg)	26±4	26±7	0.784
Visceral fat (L)	3±1	3±2	0.661
Systolic blood pressure (mmHg)	143±17	133±10	0.118
Diastolic blood pressure (mmHg)	84±7	82±9	0.621
Cholesterol (mmol/L)	4.8±1.0	3.8±1.1	0.035*
Triglycerides (mmol/L)	2±1	2±2	1.000
HDL (mmol/L)	1.4±0.5	1.3±0.3	0.865
LDL (mmol/L)	3.1±0.8	1.5±0.4	<0.001***
Qrisk^2 (%)	14±15	12±9	0.754

*denotes significance of <0.05

**denotes significance of <0.01

***denotes significance of <0.001

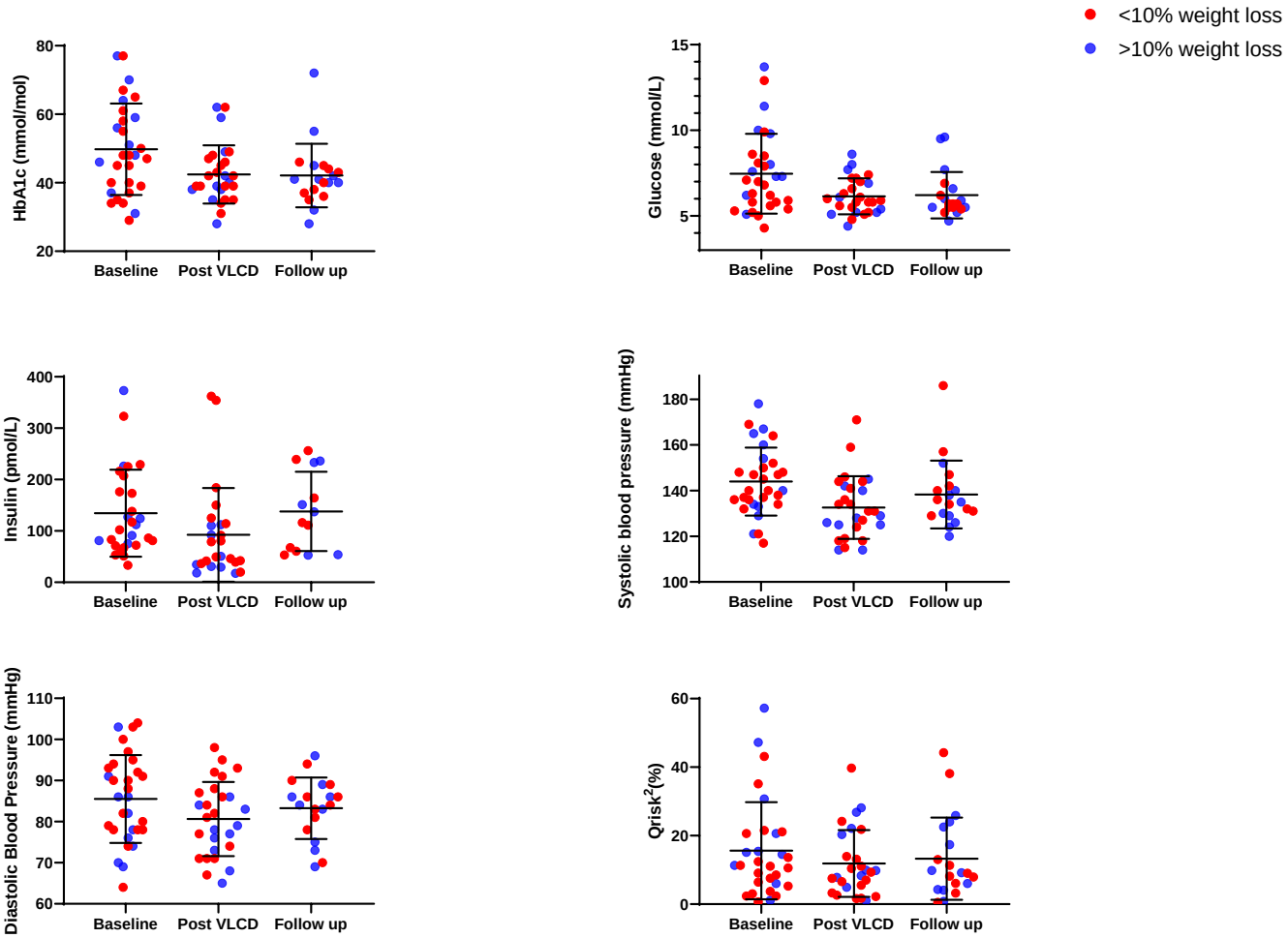


Figure 3.15. The changes in indicators of cardiovascular health at baseline, post-VLCD and follow-up in patients who lost </>10% weight loss. This Figure indicates the changes of A) HbA1c (mmol/mol), B) fasting glucose (mmol/L), C) Insulin (pmol/L), D) systolic blood pressure (mmHg), E) diastolic blood pressure (mmHg) and F) Qrisk^2 score in patients who lost </>10% weight loss.

3.5.4.7. *Quality of life*

At follow-up, patients who had lost less than 10% weight loss did not experience significantly different measures of quality of life or weight-related symptom measure (see Table 3.12 and Figure 3.16).

Table 3.12. Measures of quality of life in patients who lost </>10% weight loss at follow-up.

Variable	**<10% weight loss (n=10)**	**>10% weight loss (n=10)**	**P value**
Quality of life	55±29	56±21	0.963
Weight-related symptom measure	26±17	31±27	0.652

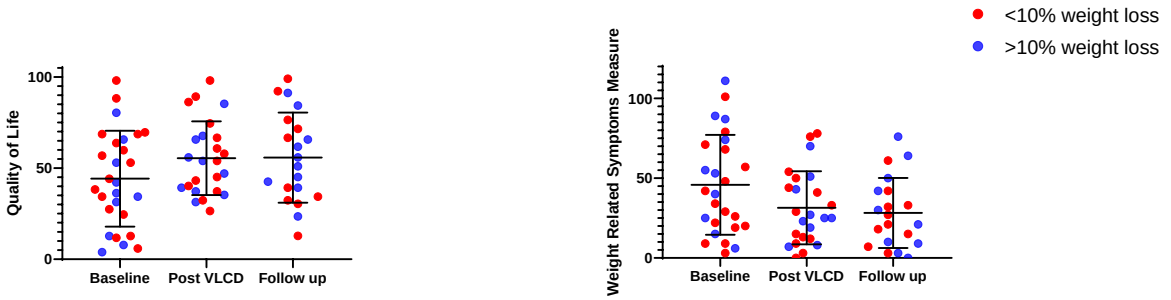


Figure 3.16. The changes in quality of life (A) and weight-related symptom measure (B) between baseline, post-VLCD and follow-up in patients who lost </>10% weight loss.

3.5.5. Exploring the correlations between weight loss and secondary outcomes

To further explore the direct relationship between weight loss (%) and measures of liver health, metabolic control, cardiovascular disease risk, and QOL, changes in variables of interest between baseline and final follow-up were correlated with weight loss (%). The distribution of datasets were assessed for normality, and either Pearsons product moment correlation or Spearmans correlation coefficient were calculated accordingly.

3.5.5.1. *Liver health*

Weight loss (%) was found to be significantly, positively correlated with changes in AST (r=0.452, p=0.045) and NAFLD fibrosis score (r=0.565, p=0.009) (see Table 3.13 and Figure 3.17). Similarly, changes in ALT and weight loss (%) were non-significantly, positively correlated (r=0.436, p=0.055) (see Figure 3.17). Although a non-significant correlation was observed, this relationship is potentially clinically meaningful, particularly as the correlation was trending towards significance. This may suggest that greater weight losses were associated with greater reductions in ALT, which would therefore correspond with improvement in markers of liver health which could potentially be of great benefit to people with NAFLD (see Table 3.13 and Figure 3.17). Similarly, Figure 3.18 highlights the correspondence between the percentage weight loss (%) and ALT (IU/L) and AST (IU/L) levels throughout the VLCD intervention.

Table 3.13. Correlations between indicators of liver health and weight loss (%).

Variable	**R value**	**P value**
AST (IU/L)	0.452	0.045*
ALT (IU/L)	0.436	0.055
GGT (IU/L)	0.335	0.149
Liver stiffness (kpa)	0.018	0.946
Fib4	0.350	0.131
NAFLD fibrosis score	0.565	0.009**

*denotes statistical significance of <0.05

**denotes statistical significance of <0.01

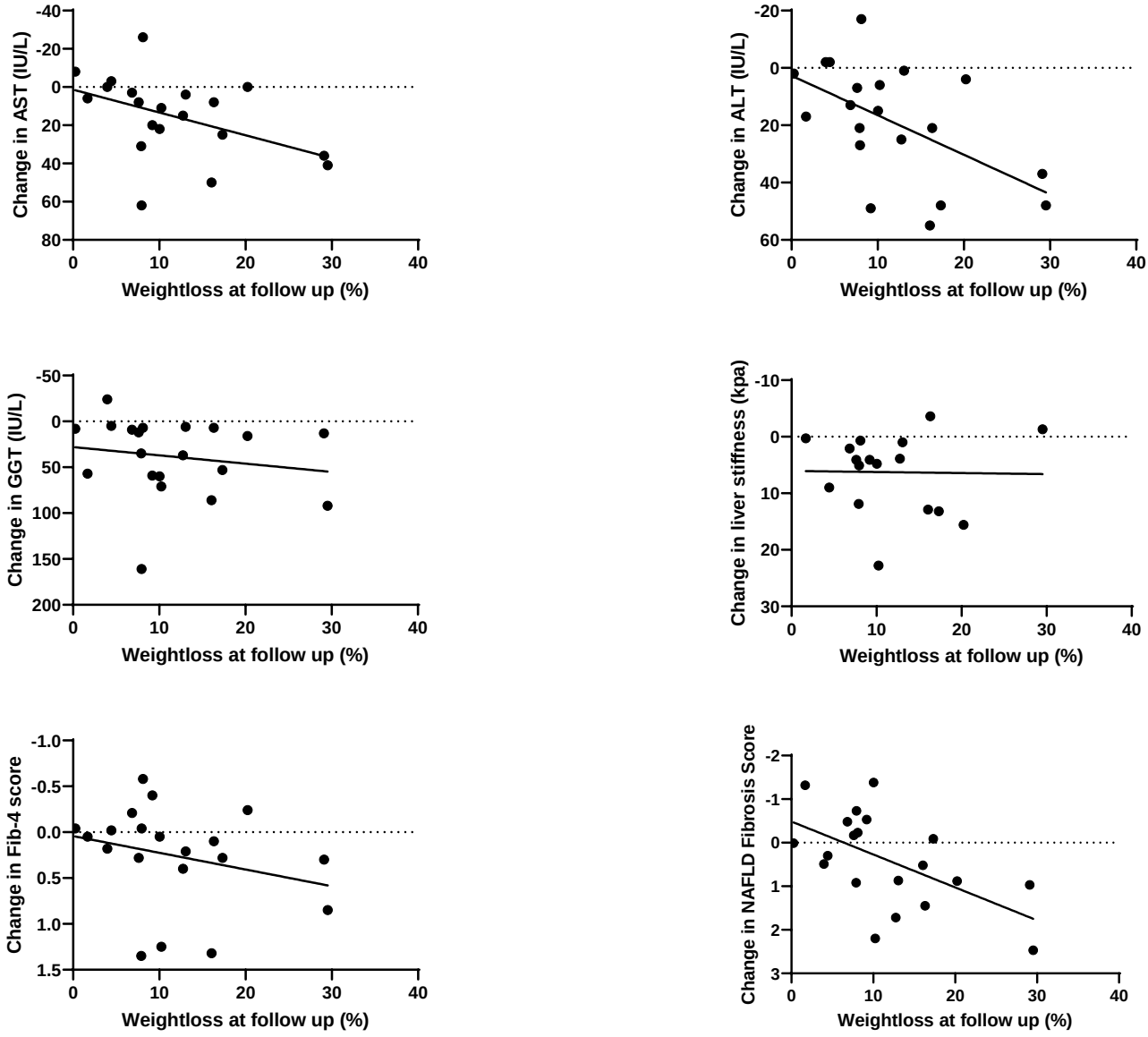


Figure 3.17. The correlations between changes in indicators of liver health and weight loss (%).

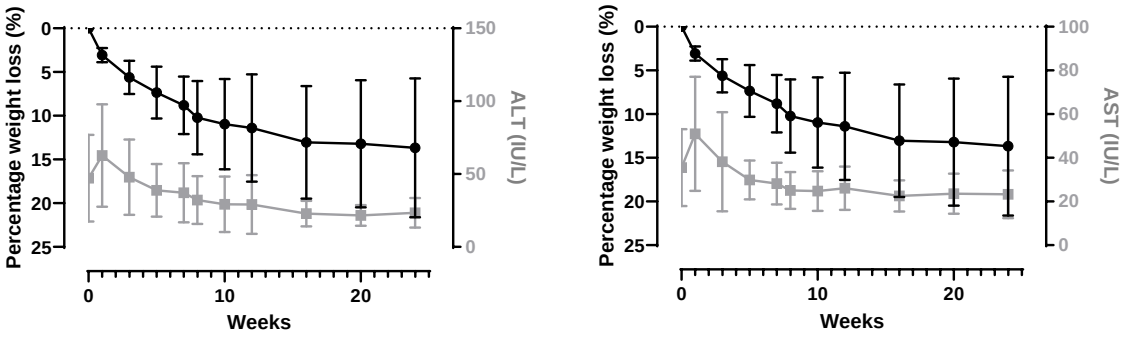


Figure 3.18. The correspondence between percentage weight loss (%) and A) ALT (IU/L) and B) AST (IU/L).

3.5.5.2. Metabolic control

Weight loss (%) was not found to be significantly correlated with changes in indicators of metabolic control (see Table 3.14 and Figure 3.19).

Table 3.14. Correlations between indicators of metabolic control and weight loss (%).

Variable	**R value**	**P value**
Fasting glucose (mmol/L)	0.220	0.365
HbA1c (mmol/mol)	0.157	0.520
Insulin (pmol/L)	0.052	0.865
HOMAIR	-0.178	0.601
HOMAB	0.315	0.346
HOMAS	-0.240	0.478

3.5.5.3. Cardiovascular disease risk

Weight loss (%) was found to be significantly, positively correlated with changes in fat mass (%) ($r=0.808$, $p<0.001$), skeletal muscle mass ($r=0.624$, $r=0.010$), visceral fat ($r=0.845$, $p<0.001$), systolic blood pressure ($r=0.544$, $p=0.016$), LDL ($r=0.590$, $p=0.026$) and Qrisk^2 ($r=0.642$, $p<0.001$) between baseline and follow-up, as shown in Table 3.15 and Figure 3.19.

Table 3.15. Correlations between indicators of cardiovascular disease risk and weight loss (%).

Variables	**R value**	**P value**
Fat mass (%)	0.808	<0.001***
Skeletal muscle mass (kg)	0.624	0.010**
Visceral fat (L)	0.845	<0.001***
Systolic blood pressure (mmHg)	0.544	0.016*
Diastolic blood pressure (mmHg)	0.163	0.503
Cholesterol (mmol/L)	0.292	0.212
Triglycerides (mmol/L)	0.272	0.247
HDL (mmol/L)	-0.280	0.232
LDL (mmol/L)	0.590	0.026*
Qrisk^2 (%)	0.642	<0.001***

*denotes significance of <0.05

**denotes significance of <0.01

***denotes significance of <0.001

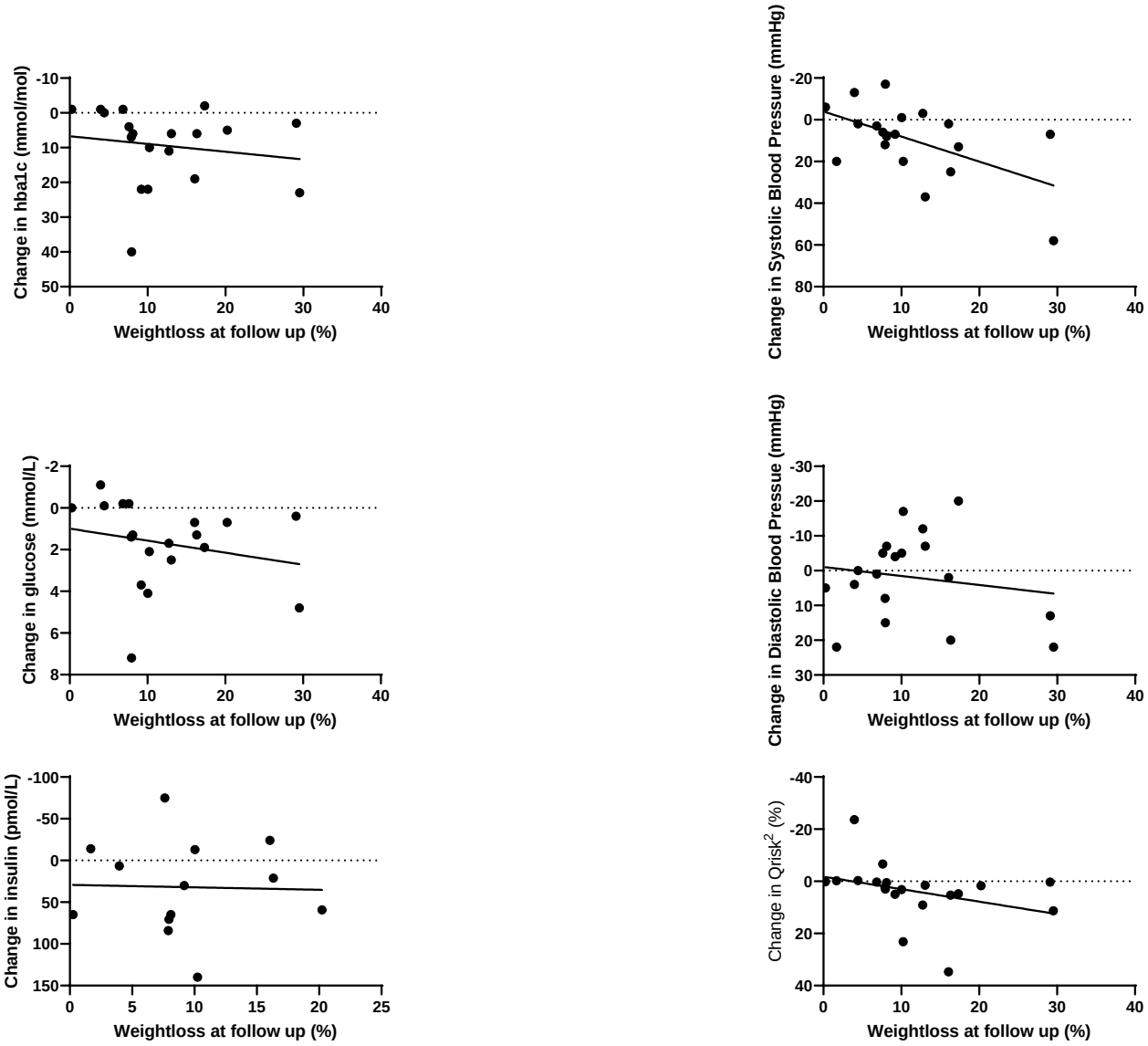


Figure 3.19. Correlations between cardiometabolic risk factors and final weight loss (%). This Figure indicates the correlations between final weight loss (%) and A) HbA1c (mmol/mol), B) fasting glucose (mmol/L), C) insulin (pmol/L), D) systolic blood pressure (mmHg), E) diastolic blood pressure (mmHg), F) Qrisk^2 (%)

3.5.5.4. *Quality of life*

Weight loss (%) was not found to be significantly correlated with changes of measures of obesity related quality of life (see Table 3.16 and Figure 3.20).

Table 3.16. Correlations between measures of obesity related quality of life and weight loss (%).

Variables	**R value**	**P value**
Quality of life	-0.161	0.497
Weight related symptom measure	0.312	0.181

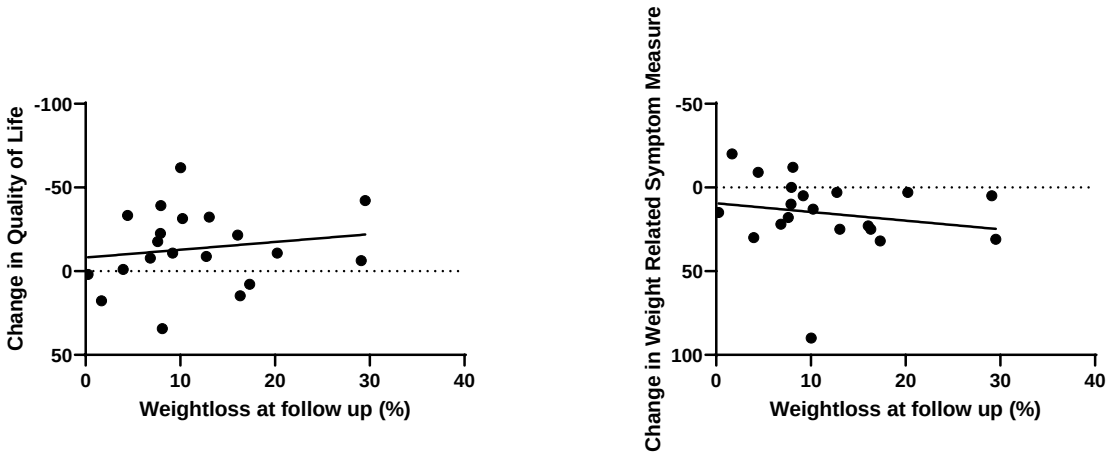


Figure 3.20. Correlation between A) QOL and final weight loss (%) and B) WRSM and final weight loss (%)

3.5.6. The effect of a VLCD on patients with advanced NAFLD and T2DM: an exploratory analyses

16 (53%) patients had T2DM at baseline (HbA1c: 59±10 mmol/mol), and within this subgroup 15 (88%) were prescribed oral antidiabetic medications. Following the VLCD intervention, mean HbA1c reduced to 46±8 mmol/mol and 6 (40%) patients reduced their oral antidiabetic medication dosage. HbA1c in patients with T2DM reduced by a mean of 13±12 mmol/mol, compared to 3±3 mmol/mol (p=0.025) in those without T2DM between baseline and follow-up. Similarly, patients with T2DM experienced a significantly larger change in fasting glucose between baseline and follow-up (2.2±2.2 mmol/L vs. 0.6±0.6 mmol/L, p=0.035). There were no significant differences in changes in insulin, HOMAIR, HOMAB, or HOMAS between patients who had T2DM and those who did not.

Figure 3.21 indicates the HbA1c of the study cohort at baseline, post-VLCD, and at follow-up. Here it can be observed that those with NAFLD but without T2DM did not experience significant changes in HbA1c, whereas those with both NAFLD and T2DM experienced a significant reduction in HbA1c. This likely indicates that the significant changes in HbA1c reported for the whole cohort (section 3.5.2, Table 3.3, Figure 3.11) are due to the significant reduction observed in patients with NAFLD and T2DM.

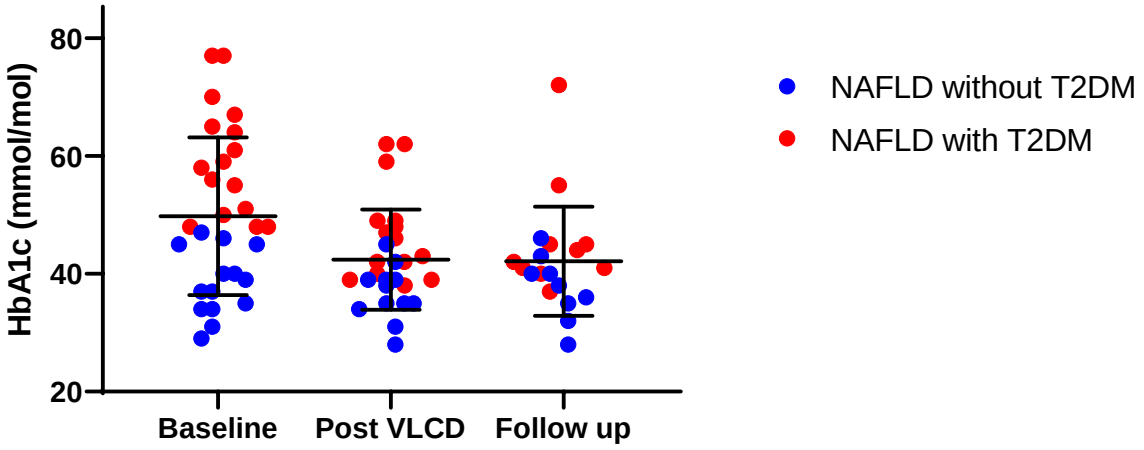


Figure 3.21. The changes in HbA1c throughout the study, where red indicates those with T2DM and blue indicates those without.

3.6. Discussion

3.6.1. Primary and secondary outcomes

Weight loss achieved through lifestyle behaviour change is currently the recommended first-line treatment for NAFLD. Previous studies have shown that, if successful, these changes can improve liver histology and reduce risk of disease progression (Vilar-Gomez et al., 2015, Promrat et al., 2010). However, few patients (10%) achieve the recommended target of sustained weight loss of >10% using standard lifestyle interventions (Vilar-Gomez et al., 2015). Therefore, alternative approaches are needed. This current study shows that a VLCD intervention is an acceptable and feasible method to enable significant sustainable weight loss in obese individuals with NAFLD. Overall, 90% of those enrolled completed the VLCD phase of the intervention and 59% of completers achieved ≥10% weight loss post-VLCD. Importantly, a large proportion of the whole cohort (34%) maintained ≥10% weight loss for at least 6-months after completing the VLCD intervention (Scragg et al., 2020). Absolute weight losses were impressive, with a mean loss of 10.3 kg at 9-month follow-up, consistent with previous studies of VLCD (Lean et al., 2019, Lean et al., 2018). This compares favourably to a study of standard clinical care (Vilar-Gomez et al., 2015). Overall these results suggest that VLCD is a viable treatment option for some patients with NAFLD to enable significant weight loss. Despite the potentially perceived drastic nature of the intervention, recruitment to the study was straightforward. Thirty patients were recruited at a single site within six months, and 67% of patients offered the opportunity to take part in the study were enrolled.

The relatively high level of uptake to the intervention (67%) was larger than previous studies exploring the use of a meal replacement based VLCD. For example, DiRECT had an uptake rate of approximately 20% (Lean et al., 2018). However, this could largely be due to differences in the mode of delivery and therefore the more resource intensive demands of the current study and the delivery through secondary care. Previous studies of VLCD have largely been conducted in individuals with obesity and T2DM and these have consistently shown that a VLCD can facilitate weight loss, and this was associated with reversal of diabetes in 46% of patients (Lean et al., 2018). To date, the impact of the VLCD specifically on NAFLD has not been assessed. Previous research has highlighted that the motivations for NAFLD patients undertaking a VLCD are likely to be different, particularly because many are asymptomatic and are not concerned about their condition (Hallsworth et al., 2019, Avery et al., 2017, Haigh

et al., 2019). In the current study, patients with fibrotic NAFLD were included because these individuals are at risk of progression to cirrhosis. Significant improvements in liver enzymes (ALT, AST and GGT) were seen at the end of the VLCD phase and this was maintained at 9-months follow-up. Previous studies assessing vitamin E and obeticholic acid showed that falls in ALT were associated with improvements in hepatic inflammation, so it is likely that improvements in liver enzymes associated with the VLCD indicate improved liver health of these individuals. In addition, liver stiffness significantly improved at 9-month follow-up providing further evidence of improved liver health.

Although NAFLD is a disease of the liver, CVD is the most common cause of death in patients with NAFLD, accounting for approximately 40% of deaths (Angulo et al., 2015). In the current study, there were improvements in the patients' cardiometabolic status following the VLCD, with significant reductions in blood pressure, cholesterol levels and 10-year CVD risk, and improved blood glucose control. These findings were consistent with previous studies of the VLCD (Leslie et al., 2016, Vilar-Gomez et al., 2015). In contrast, other drugs that have shown benefit in NASH, such as vitamin E and obeticholic acid, have not shown to have a positive effect on cardiometabolic status. Moreover, use of obeticholic acid in patients with NASH was associated with a rise in LDL cholesterol and total cholesterol levels, and a fall in HDL cholesterol within the first month of treatment (Younossi et al., 2019).

Interestingly, one week into the VLCD there was a significant rise in serum ALT and AST in patients which returned to baseline by week 4, and transaminases fell thereafter. The cause of this acute rise in transaminases was not determined. One potential mechanistic explanation may be that rapid weight loss increases lipolysis in adipose tissue resulting in high levels of circulating free fatty acids that are taken up by the liver. These free fatty acids may cause lipotoxicity in hepatocytes leading to apoptosis and cell death and a consequent rise in liver enzymes (Feldstein et al., 2004). The pathophysiology of this phenomenon requires further investigation.

At baseline, the mean BMI of the cohort was 42 kg/m^2 (morbidly obese) and this reduced to 35 kg/m^2 9-months after the intervention, meaning the majority of the cohort were still obese. Despite this, there were significant improvements in liver and cardiometabolic health in the cohort even though patients did not achieve a "normal" BMI. This is an extremely important message to relay to patients who may feel that reaching a "normal" BMI is

unachievable. A weight loss target of >10%, with appropriate support, may be a more realistic goal that can have significant health benefits. Previously there have been concerns that VLCD interventions may induce or increase sarcopenia amongst cohorts of overweight and obese patients (Yanai, 2015b). In our study there was no significant change in skeletal muscle mass after the VLCD. Although this had decreased slightly at 9-month follow-up. This highlights the importance of monitoring muscle mass closely during and after a VLCD intervention, and encouraging patients to increase their physical activity/exercise levels during the food reintroduction and weight maintenance phases and to maintain this in the long term to avoid sarcopenia.

As well as improving liver and cardiometabolic health, it would be advantageous for a treatment for NAFLD to improve QoL as previous studies have shown that patients with NAFLD report significantly impaired QoL. A recent study indicated a negative correlation between QoL and obesity, T2DM, and dyslipidaemia in a NAFLD population (Huber et al., 2019). Therefore, a treatment option that significantly reduced patients' weight to improve obesity and associated comorbidities would be worthwhile in order to improve QoL. Data have also shown that NAFLD populations are more likely to report burdens related to bodily pain, anxiety, shortness of breath, and an overall impairment in daily physical function (Golabi et al., 2016). Importantly, in the current study we found that there were significant improvements in QoL and there was a decrease in weight-related symptoms. Improvements in QoL following an intervention are very important, over and above improving liver and cardiometabolic health, because they may promote greater adherence to a treatment in the longer term as patients notice a benefit in their day-to-day life (Florez et al., 2010, Nunes, 2001). It is worth highlighting that our sample included a large proportion of patients who had previously received advice to lose weight without success. Therefore, there is a case to be made for presenting patients with VLCD as a treatment option – i.e. it may not necessarily be those who are most motivated who engage with this approach, it may be a case of preference and the desire for rapid weight loss outcomes.

A feature of the current study is that patients were not required to have a liver biopsy for inclusion in the study, which increases the widespread applicability of the findings. Patients with a clinical diagnosis of NAFLD with an indeterminate NFS or FIB-4 score were eligible. These criteria were chosen because previous studies have shown that both the NFS and FIB-

4 predict long-term outcomes, and patients with NAFLD and indeterminate or high scores have increased risk of liver-related and all-cause mortality. Therefore, these inclusion criteria are likely to have identified individuals in need of treatment for their NAFLD (Blank et al., 2020). Moreover, in contrast to many of the currently recruiting trials of pharmaceutical agents, our eligibility criteria were very inclusive and allowed patients with comorbidities, such as poorly controlled diabetes and/or morbid obesity to take part. This means that the results of this study may more generalisable to "real" NAFLD populations where patients frequently have multiple comorbidities, when compared to some studies of pharmaceutical agents.

3.6.2. Analyses of patients who withdrew from the study

The identification of a particular group of patients or an indicative variable who may be more likely to withdraw from the intervention would be of paramount importance to the delivery of the intervention in clinical practise, as this would allow those delivering the intervention to understand who may benefit from greater support. However, given the relatively small numbers of patients who withdrew from the study (three during the VLCD intervention and seven thereafter) it is challenging to undertake a sophisticated statistical analysis in order to compare this group to the initial baseline cohort or to those who remained engaged with the study throughout its entirety. Despite this, it can be seen that the population who remained engaged with the study throughout do not differ significantly from those who withdrew. Going forward, this analysis would benefit from a larger sample size in order to fully evaluate potential indicators which allude to a greater chance of withdrawing from the study.

3.6.3. Achievement of 10% weight loss

A plethora of research has identified the achievement of 10% weight loss to be of significant health benefit in patients with NAFLD and associated comorbidities, such as T2DM, obesity CVD risk, and obstructive sleep apnoea (Wing et al., 2011, Vilar-Gomez et al., 2015, Poirier et al., 2003, Lambert et al., 2014, Peppard et al., 2000, Lean et al., 2018). However, the general consensus is that all weight loss is beneficial in NAFLD patients and that greater weight losses are associated with larger clinical benefits (Peppard et al., 2000, Lean et al., 2018).

In patients with NAFLD, it has been reported that improvements in NAFLD activity score (NAS), and the individual elements of the NAS are significant following moderate (between 7 and 10%) weight loss, and even more so in those that lost more than 10% weight loss. Individual

elements of the NAS are steatosis, lobular inflammation, ballooning, fibrosis and portal inflammation. Degree of weight loss has been independently associated with improvements in all NASH-related histologic parameters. All patients who lost ≥10% of their weight had reductions of NAS, 90% had resolution of NASH, and 45% had regression of fibrosis (Vilar-Gomez et al., 2015). A major limitation of this study was the relatively low percentage of patients that managed to achieve 10% weight loss, with less than half achieving greater than 7% weight loss, and less than 30% achieving a 10% weight loss.

The presented data shows clinical, but not significant, differences in indicators of liver health at follow-up between those who had achieved </>10% weight loss. This is likely due to an insufficient sample size which lacks power for this depth of analyses, however, this analysis is purely exploratory. There were no significant differences in baseline characteristics of those who ultimately lost </>10% weight loss, with the exception of LDL (Table 3.6). GGT was significantly lower in those who achieved > 10% weight loss (47 vs.27, p=0.018). AST and ALT were trending towards significance, with levels lower in those who had achieved a weight loss of > 10%. Importantly, all measured biochemical mean scores at follow-up were considered to be normalised, according to the American College of Gasteroenterology (Kwo et al., 2017), those who had not achieved 10% weight loss were much closer to the upper accepted limit of the normal range. Total cholesterol (4.8 vs. 3.8, p=0.035) and LDL (3.1 vs.1.5, p<0.001) were significantly lower in those who had achieved >10% weight loss. Indicators of liver or cardiometabolic health following the achievement of </>10% weight loss have not previously been reported and this data is therefore novel. However, given the small sample size of the present dataset, to further explore the differing effects of 10% weight loss, a larger sample size should be used in future studies.

3.6.4. Correlations between measured variables and weight loss

As previously described, greater weight losses have previously been reported to be associated with greater improvements in indicators of liver and cardiovascular health (Vilar-Gomez et al., 2015, Poirier et al., 2003).

Previous studies have reported significant correlations between weight lost (%) from baseline to follow-up and liver enzymes. Vilar-Gomez *et al.* observed positive, significant correlations between weight loss (%) and AST (r=0.22, p<0.001), ALT (r=0.23, p<0.001) and GGT (r=0.150, p<0.001) (Vilar-Gomez et al., 2015). Conversely, the presented data only reported significant,

positive correlations between AST and weight loss (%) (r=0.452, p=0.045). Correlations between weight loss (%) and ALT and GGT were positive but were not considered to be significant, despite the correlation with ALT likely being of clinical importance (r=0.436, p=0.055).

Vilar-Gomez *et al.* also investigated correlations between weight loss (%) and some indicators of cardiovascular health- such as total cholesterol and triglycerides. Significant positive correlations were observed between weight loss and cholesterol, but not triglycerides (Vilar-Gomez et al., 2015). The present data is novel in its extensive range of variables whose correlates with weight loss (%) have been assessed. As indicated in Tables 3.13 and 3.14 and Figures 3.17 and 3.19, changes in NFS, QRISK2, systolic blood pressure and LDL were positively, significantly correlated with weight loss (%). Further work is required to assess correlations with over a greater period of time and within a larger cohort. This would allow for further, more in-depth analyses of the correlates and subsequent greater understanding of potential predictors of significant weight loss. As identified in Figure 3.13, the present data identified early weight loss (weight loss achieved three weeks into the intervention) as being significantly correlated with achieving 10% weight loss, suggesting that adherence and subsequent success of a VLCD could be identified early within the intervention. This highlights an important period of time in which good adherence should be advocated as much as possible to patients.

3.6.5. The effect of a VLCD on patients with NAFLD and T2DM

Previous work has identified the efficacy of a VLCD (800kcal/day) within a T2DM cohort (Leslie et al., 2016, Lean et al., 2018). Given the underlying pathophysiology of T2DM and NAFLD (see sections 1.2.2 and 1.2.3), there is likely a prominent crossover of patient characteristics between the presented data and that obtained from the DiRECT study (Lean et al., 2019). Indeed, within our cohort, 53% of all patients at baseline had clinical diagnoses of T2DM. A significant finding from DiRECT was the measurement of liver fat at baseline, post VLCD and at follow-up (12 months). The primary outcome measure of our study was to evaluate the feasibility of recruiting NAFLD patients and achievement of 10% weight loss. The nature of the study was designed to map onto current clinical care to observe the feasibility effect within current standard practise, therefore, liver fat was not measured as part of this study. The DiRECT study measured mean liver fat at 16% at baseline and at approximately 3% post

VLCD and at 12 months follow-up. Given the interlinked pathophysiology of the two conditions, it is likely that liver fat changes would be similar in our cohort. Going forward, this is one of the prominent variables to be measured in a NAFLD cohort. So far, this has not been done and it would provide valuable insight into the mechanisms of NAFLD resolution following a VLCD.

3.7. Study limitations

The data presented the findings of a feasibility study designed to assess acceptability and feasibility of the VLCD intervention for achieving >10% weight loss and associated study procedures. A primary limitation within this study is the lack of formal reporting of adherence. At each study visit patients were asked about any deviation from the VLCD protocol since the previous study visit. The dependency on patients to honestly self-report deviations from the protocol meant that no formal analyses could be undertaken on adherence, although weight loss was largely considered to be a surrogate marker for adherence. Given the mean BMI of this cohort and their subsequent daily energy requirements, it was thought that 800 kcal/day would provide sufficient caloric deficit to induce weight loss. Similarly, another limitation is the lack of reporting of fidelity measures. However, as previously discussed, delivery of the intervention was standardised in that the structure of every study visit was the same and largely delivered by the same two members of the research team who had both received the same training with regards to delivering a VLCD intervention.

Similarly, while best practise would stipulate that this study protocol and its associated primary outcomes should be prospectively registered to a trial registry, this trial was registered during data collection and in advance of data analysis. Prospective registration would have served to increase trial transparency and subsequent research integrity by reducing potential publication bias and selective reporting. Similarly, this would have served to provide greater opportunities for researcher collaboration and reducing research duplicity. Due to the nature of this research being undertaken within the constraints of a PhD (at research training programme), information relating to choosing the appropriate trial registry was not conveyed prior to recruitment start date and therefore registration occurred during data collection but prior to data analysis. In order to maximise research

integrity in light of the delayed registration, no data analysis occurred until after the trial was suitably listed on the ISRCTN register. The ISRCTN register was chosen due to its publicly accessible and free to access data. Importantly, it is the preferred partner of the UK Department of Health and Social Care and facilitated the easy adoption of the study to NIHR Clinical Research Network Portfolio (CRN). This study was adopted to the NIHR CRN Portfolio to further maximise research transparency.

Considering that this was a feasibility study, the results of the secondary outcomes should be considered exploratory, i.e. the study was uncontrolled and not powered to detect changes in secondary outcomes. Secondly, non-invasive tests rather than liver biopsy were used for inclusion of patients and monitoring of liver outcomes in the study, and as such we were only able to report a global assessment of liver health using liver enzymes and liver stiffness measurement and we were unable to report which elements of the clinical physiology of NAFLD had improved; steatosis, hepatic inflammation or fibrosis. There have been issues with regards to reliability of using Fibroscan to measure liver stiffness in obese patients reported (Castéra et al., 2010), but this represents current clinical practice. Thirdly, a significant proportion of patients (33%) were lost to follow-up at 9-months follow-up and data on their outcomes was limited (although follow-up data for weight was available from recent clinical visits). This meant it was therefore unable to accurately describe all "irrespective of completion" outcomes for the whole population. Furthermore, if all outcomes for patients were included, overall cardiometabolic and liver outcomes may have been less pronounced. Fourthly, patients on insulin for treatment for diabetes were excluded, which represents a significant proportion of the NAFLD population. The decision to exclude patients on insulin was taken to ensure safety because rapid weight loss can cause hypoglycaemia. Fifthly, one of the primary objectives of this study was to assess the proportion of patients willing to undertake the VLCD as a treatment for NAFLD, however, there is likely to be a selection bias with clinicians potentially approaching more motivated patients. This could have contributed to the successful outcomes. Finally, the length of VLCD phase was not standardised and patients could extend the intervention from 8 to 12 weeks if there were mitigating circumstances, and this allowed some patients to optimise their weight loss outcomes. Given that intervention effects started to wear off towards the end of the follow-up period, it is likely that 6-months post-intervention follow-up is insufficient to assess weight loss

maintenance. Further work is needed to assess outcomes in a larger cohort in a “real world” setting using VLCD interventions of varying length.

3.8. Conclusions

Overall this study showed that delivery of a VLCD is feasible, acceptable and a potential treatment option for some individuals with NAFLD, with a significant proportion of those who complete the intervention achieving >10% weight loss and maintaining it at 9-month follow-up. Importantly, the weight losses achieved in this study exceed those reported for standard clinical care. Improvements were also observed in liver health, metabolic control, cardiovascular risk, and QoL in those completing the intervention at 9-months follow-up. A VLCD intervention offers a holistic treatment option that could be incorporated as part of clinical care for some patients with NAFLD.

4. Factors associated with engagement and adherence to a very low calorie diet to achieve significant weight loss in patients with advanced non-alcoholic fatty liver disease: A qualitative interview study

Abstract

Objective: Clinical guidelines recommend weight loss to manage non-alcoholic fatty liver disease (NAFLD). However, the majority of patients find weight loss a significant challenge. We identified factors associated with engagement and adherence to a very low calorie diet (VLCD) as a treatment option for NAFLD.

Design: 23 patients with advanced NAFLD who were enrolled in a VLCD (~800 kcal/day) were interviewed. Qualitative interviews were audio recorded, transcribed verbatim and thematically analysed.

Results: Adherence to the VLCD intervention was high - 53% of all patients achieved ≥10% weight loss, 63% achieved ≥7% weight loss and 77% achieved ≥5% weight loss. A desire to achieve rapid weight loss to improve liver health and prevent progression was the most salient facilitator to engagement. Early and significant weight loss; accountability to clinicians providing support; and regular appointments with personalised feedback were facilitators to continued engagement and adherence. The desire to receive positive reinforcement from a consultant was a frequently reported facilitator to adherence. Practical and emotional support from friends/family members was critically important external to the clinical setting. Irregular working patterns/shift work that prevented attendance at appointments was a barrier to adherence and completion of the intervention.

Conclusions: Engagement and adherence to a VLCD in patients with advanced NAFLD relies on early and rapid weight loss, personalised feedback and positive reinforcement in the clinical setting combined with ongoing support from friends and family members. Findings support those identified in patients with type 2 diabetes who participated in a VLCD to achieve diabetes remission and highlights the importance of intensive behavioural support during the early stages of a VLCD to promote longer-term adherence.

4.1. Introduction

Given the absence of approved pharmaceutical agents for the treatment of NAFLD, lifestyle modification, typically weight loss, is the primary recommended therapy (NICE, 2016c, Chalasani et al., 2012). A weight loss goal of 10% of initial body weight has been recommended for patients with advanced NAFLD (Vilar-Gomez et al., 2015, Dyson et al., 2014). Indeed, research has shown that 90% of patients losing more than 10% of their initial body weight had resolution of steatohepatitis after 1 year, and 81% showed improvements in fibrosis (Vilar-Gomez et al., 2015). However, weight loss maintenance was highlighted as an issue with only 10% of patients maintaining 10% weight loss after one year. In patients with NASH, weight loss has shown strong associations with improvements in NAFLD activity score (NAS) (Promrat et al., 2010). These findings highlight the importance of providing an intervention that is widely acceptable to a larger proportion of individuals with NAFLD to elicit significant weight loss.

Very low calorie diets (VLCDs) have demonstrated to be a viable treatment strategy for people with type 2 diabetes mellitus (T2DM) (Steven et al., 2016). Research has shown that VLCDs are effective for achieving substantial weight loss, with high levels of adherence and low levels of attrition in overweight and obese people with T2DM (Rehackova et al., 2016). A randomised controlled trial of a VLCD conducted in primary care involving patients with T2DM found that 24% of those in the intervention group lost ≥15 kg, and mean body weight fell by 10 kg at one year follow-up (Lean et al., 2018). Another study showed that 45% of obese patients undertaking a 12-week VLCD maintained ≥10% weight loss at one year follow-up (Jebb et al., 2017). Research suggests that VLCD may also have a positive impact on fatty liver; patients with T2DM (Lim et al., 2011) treated with VLCD had a reduction in liver fat from 13% to 3%. Despite these findings, the VLCD approach has not been formally assessed as a treatment strategy for NAFLD.

The totality of these changes could be beneficial to patients with NAFLD in reversing liver disease, halting disease progression or reducing other obesity-related risk factors. While this has been shown to be a successful and viable treatment option for patients with T2DM, given the proportion of NAFLD patients with asymptomatic disease burden, it is important to establish whether uptake, engagement and adherence can be achieved in this population.

Previous studies evaluating the feasibility and efficacy of delivering a VLCD in the form of meal replacement products have retrospectively assessed barriers, facilitators and motivations towards uptake, engagement and adherence (Astbury et al., 2020a, Rehackova et al., 2017). Patients with obesity and T2DM have reported that the desire for weight loss has been the primary motivator to uptake, and that this coincided with patient expectation; most patients expected to achieve significant weight loss. Similarly between cohorts, rapport and the support of the clinical/ research team members facilitated adherence (Astbury et al., 2020a, Rehackova et al., 2017). Furthermore, the regimented simplicity of the VLCD in both obese and T2DM cohorts facilitated adherence. Patients reported in both instances that the intervention was challenging but that the overall positive outcomes by far outweighed any negatives outcomes such as minor adverse events, or feelings of missing out on social situations (Astbury et al., 2020a, Rehackova et al., 2017). Given the physiological similarities and overlapping features between T2DM, obesity and NAFLD, it is likely that the VLCD will have a similar effect on all three cohorts. However, given the relatively low awareness of NAFLD, it is likely patient priorities and motivators may differ and therefore it is essential to assess the acceptability of such a drastic intervention in a NAFLD cohort to inform on whether this unique cohort consider this to be an acceptability therapy.

Eliciting lifestyle changes in NAFLD patients can be challenging (Bellentani et al., 2008). Previous research has reported that a large proportion of NAFLD patients lack motivation or readiness to change lifestyle behaviours (Centis et al., 2013). However, evidence-informed information communicating the importance of lifestyle and the role lifestyle behaviours, specifically diet, play in the manifestation and progression of NAFLD is arguably insufficient to elicit behavioural change (Centis et al., 2013). A large proportion of patients with NAFLD typically fall into the 'hard to reach' population, and therefore often are reluctant to change and show resistance in regards to acquiring 'positive lifestyle behaviours' (Zwolinsky et al., 2013). Consequently, this population, typical of NAFLD cohorts, are frequently unaffected by typical health promotion activities (Zwolinsky et al., 2013, Poortinga, 2007). It is therefore important to thoroughly evaluate the acceptability of new alternative interventions in NAFLD cohorts, in order to maximise uptake and adherence going forward.

A qualitative study was conducted with individuals with advanced NAFLD participating in a feasibility study of a very low calorie diet (VLCD) that aimed to initiate and maintain 10%

weight loss. Specifically, the qualitative study aimed to identify barriers, facilitators and motivations towards uptake, engagement and adherence to the 8-12 week VLCD (~800 kcal/day). This study hypothesised that the VLCD intervention (as described) would be acceptable to patients with advanced NAFLD.

4.2. Methods

This study was approved by the North East - Newcastle & North Tyneside 1 Research Ethics Committee (REC reference: 18/NE/0179). All participants provided written informed consent prior to participation.

4.2.1. Intervention

The intervention involved three phases. Phase 1 (weight loss): Participants were prescribed an 8-week VLCD (~800 kcal/day) that consisted of meal replacement products (Optifast, Nestlè Health Science) provided free of charge. In addition, they were encouraged to eat three portions (240g overall) of non-starchy vegetables and drink at least two litres of water or calorie-free beverages each day. One-to-one support was provided weekly throughout this first phase via telephone calls, emails and/or face-to-face appointments (tailored to meet individual needs) to maximise adherence to the protocol and to minimise drop out. Participants were given scales to weigh themselves at home if required**.** Dietary compliance was monitored by change in body weight as reported at the clinic. Patients were asked to maintain their usual daily activities during the VLCD and not to increase their physical activity levels. In the event that compliance with the VLCD was not achieved throughout the 8-week period due to factors such as hospital admission, the intervention was extended for an additional four weeks, to a maximum VLCD time period of 12 weeks. Phase 2 of the intervention involved reintroducing participants to normal eating over a 4-week time period by replacing one meal replacement product with normal food in the first two weeks and two normal meals during weeks 3 and 4. If desired, this phase was extended to 6-weeks to help manage individual needs. Participants were given information on portion size using a “Carb and Calorie Counter” manual (Cheyette, 2010, Cheyette, 2017) and individualised dietary advice was provided using a food exchange model. The goal was to limit energy intake to individual requirements to maintain weight and participants received support to overcome

behavioural barriers (e.g., resisting temptation). Patients were advised to monitor their weight weekly at home and were encouraged to monitor their caloric intake. To achieve this, each participant was given two books containing low calorie meal plans, recipes and snack ideas. Throughout the intervention patients were able to contact a member of the research team with any questions or to report any adverse events.

4.2.2. Design

A qualitative study was conducted with those taking part in the VLCD pilot study (as described in Chapter 3). Semi-structured one-to-one interviews were conducted immediately following completion of the VLCD intervention to identify barriers and facilitators to the uptake and adherence to the intervention, motivations for taking part and to explore ways in which the intervention could be optimised (e.g., whether any additional information or support was required). See appendix E for the topic guide used to guide the semi-structured interviews. The aim was to use responses to better understand how the intervention could be integrated into routine clinical care if it demonstrated to be acceptable and feasible.

4.2.3. Participants

Patients were recruited by hepatologists or other health care professionals from a specialist NAFLD clinic at a tertiary hepatology centre, The Newcastle upon Tyne Hospitals NHS Foundation Trust or the CRESTA clinic, Campus of Ageing and Vitality, Newcastle upon Tyne. Both cover a broad geographical area in Northern England. Eligibility criteria are described in Chapter 2.2. All participants (N=30 at baseline, n=27 completed VLCD) from the pilot study were invited to take part in an interview. The aim was to recruit a purposive sample of at least 20 participants in order to achieve maximal variation (e.g. gender, those who struggled to adhere to the intervention, those who achieved maximal weight loss) and data saturation. Participants were invited to take part initially in person, given time to consider participation, and subsequently followed up by email or telephone to discuss participation.

4.2.4. Study procedure

Semi-structured interviews were conducted with the support of a Chartered Health Psychologist with expertise in health behaviour change and qualitative research methods (see appendix E for the topic guide). This member of the research team had met none of the patients prior to commencing the interview. In attendance at the interviews were the participant, an invited member of the participants family in three occasions and the researcher. Interviews were conducted in a consultation room at the Freeman Hospital, Newcastle upon Tyne, following the study visits of each participant and corresponded with the completion of the VLCD intervention.

4.2.5. Materials

The research team developed a topic guide (appendix E) to facilitate discussions with participants. Topics included motivators for taking part; expectations and perceived barriers prior to participation; barriers to adherence and strategies used to overcome barriers; support requirements to maximise adherence to the intervention; and the roles of participants social and work environments in the context of intervention adherence. All questions were open ended and prompts were used to facilitate a more in-depth discussion in order to fully explore the participants' views. All interviews were audio recorded and transcribed verbatim.

4.2.6. Methodological Quality and reporting

This qualitative study was conducted in accordance with the consolidated criteria for reported qualitative research (COREQ) in order to maximise methodological quality and transparency (Tong et al., 2007) (appendix K). To reduce bias from responders, two researchers were used to conduct the interviews. Both researchers read, re-read and coded all transcripts, and discussed coding to agree final thematic labels.

4.2.7. Data analysis

Data were analysed using thematic analysis (Braun and Clarke, 2006). To maximise methodological quality and trustworthiness of the findings, the analysis procedure was as follows: all interview transcripts were independently read, and a Chartered Health Psychologist re-read following initial reading. Both researchers independently coded segments of the data with reference to the first three interview transcripts to develop a coding strategy and generate preliminary themes and sub-themes; and following discussion, the same researchers agreed a preliminary group of themes and sub-themes. This process was repeated with the remaining twenty interview transcripts and both researchers agreed a final set of themes and sub-themes that best conveyed the data set following an in-depth discussion. Supporting direct quotes from participants were applied to the agreed themes and sub-themes.

Where appropriate quantitative data was assessed in order to substantiate qualitative data obtained from the thematic analysis, Pearson's product moment was used to assess to correlations. As previously described in section 2.13, all data was assessed for normality using Shapiro-Wilk.

4.3. Results

Twenty-three participants agreed to take part in a semi-structured interview. Interviews lasted between 15 and 46 minutes (mean 28 (±9) minutes). The average age of participants was 56 years and average baseline BMI was 40 kg/m^2 (see Table 4.1). Length of time since diagnosis of NAFLD ranged from 1 month to 9 years. Following the 8-12 week VLCD, 70% of those interviewed had achieved weight loss of greater than 10%. These baseline characteristics closely matched those of participants taking part in the VLCD intervention pilot study (i.e., the whole participant group), showing good representation.

4.3.1. Recruitment and retention

As previously discussed in greater detail in Chapter 3 (Section 3.5.2), this study was fully recruited to the target sample size (n=30) within 6 months from a single centre. Briefly, 67%

of patients approached were enrolled into the study, suggesting that a large proportion of patients who are eligible are motivated to take part in a VLCD intervention. Furthermore, of the 30 who were enrolled into the study, 27 (90%) completed the 8-12 weeks VLCD (16 completing 8 weeks and 11 completing 12 weeks), and 20 (67%) completing the study until the 9 months follow up. Mean weight loss, when analysed for all patients irrespective of completion, was 9.7% (11.3 kg) and 8.9% (10.3 kg) post-VLCD and at follow up, suggesting good adherence.

4.3.2. Major and minor themes

Table 4.1. Baseline characteristics and weight loss achieved immediately post-VLCD in patients who partook in interviews.

Variable	
Age (years)	56±11
Gender (m/f)	15/8
BMI (kg/m^2)	40±7
Weight (kg)	113±19
Time since diagnosis (months): mean median	25±31 12 (1-113)
Weight loss: absolute (kg) Percent of initial body weight (%)	14±13 12±5
Deprivation score: absolute quintile	28±20 3.3±1.5
Achieved 10% weight loss	16/23 (70%)

Table 4.2: A summary of themes and sub-themes derived from thematic analysis of one-to-one semi-structured interviews

Theme	**Sub-theme**	**Direct quotes**
Desire to achieve rapid weight loss to improve liver health	An opportunity to lose weight quickly was a significant motivator to uptake	"The idea of quick weight loss…that appealed" (Male, age 60. Weight loss achieved: 14 kg) "I think it was the fact that it was short term- quick and fast" (Female, 64. Weight loss achieved: 8 kg)
	Knowledge that weight loss could improve liver and diabetes related health was important	"It wasn't about vanity, you know? I just want to be healthier and live longer – that is what I am doing this for" (Female, 64. Weight loss achieved: 11 kg) "If I have eaten rich food you can feel a reaction almost from your liver. And I just wanted to feel better about that, I just wanted to feel healthier around that" (Male, 54. Weight loss achieved: 24 kg)
Accountability to clinicians providing support facilitated adherence	Regular clinic visits with personalised feedback promoted continued engagement	"The fact that I am coming to see you on a weekly basis, or a fortnightly basis, it has kept me focussed." (Male, aged 41. Weight loss achieved: 15 kg) "Definitely, I think the main thing is the visits" (Male, aged 31. Weight loss achieved: 4 kg)

		"I suppose it's a bit like being at school, isn't it, and sort of saying, "When I go back and get weighed I want them to be pleased with me." (Female, aged 55. Weight loss achieved: 11 kg) "I would have cheated without the support" (Male, aged 68. Weight loss achieved: 16 kg)
	The desire to receive positive reinforcement from a consultant or clinical team member promoted adherence	"I like to come in every couple of weeks, just to, the talking is helping." (Male, aged 56. Weight loss achieved: 20 kg) "Seeing the surgeons face with a big smile. I walked in and he said 'you've made my afternoon'" (Male, aged 56. Weight loss achieved: 20 kg)
The structured nature of the VLCD made it easier to adhere		"Not having to think about what to eat or what to cook. It made it so much easier because I've got such an erratic lifestyle" (Female, aged 55. Weight loss achieved: 11 kg) "It is more regimental. It is laid out clearly for me, and I can follow it easily. And with the advice I have been given as to what other bits of recipes I can do I found it very,

Practical and emotional support from friends, colleagues and family members promoted adherence		very easy." (Male, aged 72. Weight loss achieved: 17 kg) "You do need a bit of your family to help you… if I was on my own, it would have been really, really hard." (Male, aged 61. Weight loss achieved: 12 kg) "The people I work with… they were really good, and they would bring food in, but they would eat it when I was away from the desk" (Female, aged 54. Weight loss achieved: 23 kg)
Early and significant weight loss promoted continued engagement and adherence		"I didn't think I would last in the first week….I got weighed, and then I'd lost all that weight in the first week, it gave me an incentive to continue with it." (Female, aged 54. Weight loss achieved: 23 kg) "In the first few weeks the motivation was seeing that I had lost a reasonable amount of weight pretty rapidly" (Male, aged 60. Weight loss achieved: 3 kg) "After the first initial five days, when I had lost all that weight, I was like, 'Yes, this, this is working I can really do this" (Female, aged 55. Weight loss achieved: 11 kg)

Working patterns make adherence to the VLCD difficult		"If I was in a position where I could work nine till five or you know regular hours, same hours day after day, if I was in a good pattern I'd have no problem" (Male, aged 60. Weight loss achieved: 14 kg) "I am really quite good during the week, unless I am having to work away and that makes it more difficult... I suffered where it was really difficult in work situations." (Male, aged 60. Weight loss achieved: 3 kg)

An opportunity to lose weight rapidly incentivised patients to take part

The opportunity to achieve rapid weight loss, specifically to improve liver health and diabetic control was reported by participants as a significant motivator to completing a VLCD. A minority of participants reported a desire to lose weight to improve other health-related conditions including musculoskeletal pain and breathing difficulties that they felt were exacerbated by excess weight. It became apparent that advocacy of weight-loss by a clinician, specifically a consultant made use of a VLCD to achieve weight loss feel 'more about health' and 'less about vanity'. As such, it was clear that health was an important motivator… *I just want to be healthier and live longer, that is what I am doing this for"*.

The majority of participants interviewed reported that rapid weight loss was more appealing than steady weight loss over a longer period of time: *"I think it was the fact that it was short term- quick and fast"*. In relation to this, participants reported having tried a variety of weight loss approaches unsuccessfully, therefore, the offer of a rapid weight loss solution that could improve health, and was supported by medical and healthcare professionals facilitated uptake of the intervention.

Accountability to healthcare professionals promoted adherence

Accountability to the clinical team promoting use of the intervention and supporting adherence to it emerged as a common and important facilitator to adherence. Specifically, accountability towards the initial referring physician was reported: "*Seeing the doctors face last Friday with a big smile on his face…he says: "You've made my afternoon."…and seeing him happy".*

Accountability towards those delivering the intervention was consistently reported as a facilitator to adherence: *"The spur of being on a diet, a sort of regime… you think 'oh, I can't let anybody down, you know'"; I suppose it's a bit like being at school, isn't it…', 'When I go back and get weighed I want them to be pleased with me'* and "*I would be saying to myself, whenever I have felt like picking, 'no, I am going to see [member of the team]… you know?"*.

The intervention was easy to follow

Ease of following the VLCD was frequently reported as a facilitator to adherence. The meal replacements played an important role by providing structure. Participants reported *"not having to think about what to eat or what to cook*". This was reported as helpful to overcome the challenges of a busy lifestyle. Additionally, meal replacements provided flexibility for those who were required to travel for work and during holidays *"I just left a few of them at work so that I didn't even have to remember to take them in with me".* Consistently the VLCD was reported as *"simple"* and something that *"didn't require much thought"*: *"It is laid out clearly for me, and I can follow it easily. And with the advice I have been given as to what other bits of recipes I can do, I found it very, very easy."*

Regular visits with positive feedback from the clinical team provided motivation to continue

Regular visits to the hospital to discuss progress and receive feedback was reported to be a key facilitator to adherence: *"I would have cheated without the support [of a healthcare professional].* Participants reported being motivated and encouraged by feedback and positive reinforcement from healthcare professionals and clinicians *"Seeing the surgeons face with a big smile.* I walked in and he said *'you've made my afternoon".*

Practical and emotional support from friends, colleagues and family members was instrumental to adherence

A need for support outside of hospital visits was reported to be critically important to overcome everyday barriers to adherence. Participants referred to work colleagues and family members making the diet easier to manage on a daily basis *"The people I work with… they were really good, and they would bring food in, but they would eat it when I was away from the desk"*. Emotional support from family members was identified as having a major influence on adherence throughout the intervention: *"You do need a bit of your family to help you… if I was on my own, it would have been really, really hard"*. Other examples of practical and emotional support included family members also restricting their caloric intake *"My wife's been the biggest supporter… she's been eating the same amount of calories as me"* and *"the help from my sister… we were always phoning each other up and she say, "Oh, I am starving." And I would say, "Oh just keep going, you will soon get over it." And I would do the same to her, so that helped.* Similarly, practical support was provided by family members who prepared suitable food or monitored their family members adherence *"They'll say, "Give me a look at your book, to see if you've been cheating."* And *"she will go shopping and get me fresh prawns, fresh fish…more vegetables and buy stuff for stir-fry's, get me water because I'm always drinking".*

Rapid early weight loss was a motivator to adhere to the intervention

Rapid weight loss during the first week of the intervention in particular was reported to be an important motivator for the majority of participants, and a consistent facilitator to adherence. Specifically, participants reported that early weight loss kept them going throughout the challenging first week of the intervention: *"I didn't think I would last, in the first week. I think, when I got weighed, and then I'd lost all that weight in the first week, it gave me an incentive to continue with it.* Participants also expressed surprise at seeing weight loss results so quickly: *"I didn't think I would lose weight in the first week, but then I did."* suggesting that the intervention exceeded their expectations.

Shift work makes adherence to the VLCD difficult

Barriers to adherence to the VLCD were highly individual. They included physically demanding jobs and family members providing temptation. However, the most consistently reported barrier identified was irregular working patterns, specifically shift work: *"If I was in a position where I could work nine till five…, regular hours, same hours day after day, if I was in a good pattern I'd have no problem"*. It became apparent that despite the structured nature and perceived flexibility of the intervention, participants who did not have a regular working pattern, or those who could not leave work to attend study/intervention visits struggled to adhere. Findings suggested that shift work and lack of support from the study/clinical team hindered planning and participants did not develop skills to overcome challenges, for example food temptations and the ability to deal with set-backs and fatigue.

4.3.3. Association between contact time and adherence

The number of study visits attended, used as a marker of contact time, was identified to be strongly positively associated with adherence (IOC weight loss as a percentage of body weight). Specifically, number of study visits attended throughout the VLCD intervention ($r=0.569$, $p=0.001$) was less strongly associated with adherence than number of visits attended through the overall study, inclusive of the weight maintenance/ follow up period ($r=0.637$, $p<0.001$).

4.4. Discussion

The VLCD for NAFLD was reported by participants to be largely acceptable to those who enrolled to study and easier than expected to adhere to. The qualitative data collected generated six themes and four sub-themes that highlighted a number of factors associated with uptake, engagement and adherence. The most salient facilitator to uptake was the desire to achieve rapid weight loss to improve liver and diabetes-related health. Participants emphasised that they would not have been so willing to take part if the weight loss strategy was slow and gradual weight loss. Importantly, health rather than body image was the driving

force for weight loss, highlighting the importance of communicating the specific health benefits of the VLCD tailored to individual patient needs. Factors associated with continued engagement with the intervention included accountability to the clinicians providing the support. In this regard, participants highlighted that regular clinic visits with personalised feedback encouraged them to engage, and their desire to receive positive reinforcement, specifically from their consultant was highly influential and promoted adherence. The structured nature of the VLCD made adherence easier for the majority of the participant group. This removed the decision-making process around food choices and was practically useful for work and some social events, although those who worked irregular shift patterns reported adherence as problematic. However, this was linked to their inability to attend clinic visits, and as such they didn't acquire new self-regulation skills or receive support. Outside of clinic visits, practical support from friends and family members was reported to be a salient facilitator to adherence. Many participants reported that they might not have completed the VLCD without the support they received at home. Interestingly, early and significant weight loss was linked with adherence, i.e. those who achieved the greatest weight loss during the first 1-2 weeks were more likely to complete the intervention and this was reflected in the qualitative data generated, highlighting the importance of intensive support during the early stages of the intervention to maximise weight loss; this was also reported within the DiRECT trial (Rehackova et al., 2020). As well as facilitators to uptake, engagement and adherence, a number of barriers were identified. These included irregular working patterns, in particular shift work. While the VLCD was acknowledged to be flexible and easy to use in the work place, the feedback and support provided during study visits were active intervention ingredients that impacted on adherence. Participants with physically demanding jobs reported feeling that the meal replacements did not provide sufficient energy requirements and this affected adherence. While family members and friends were considered to be a facilitator to adherence, they were also reported to be barriers by introducing temptation to foods. To overcome perceived barriers, participants employed multiple behavioural regulation strategies including; goal setting; planning, avoidance of food, including self-distraction and planning for difficult social situations. These strategies were positively reinforced during study visits which helped to embed them in to the everyday lives of participants. Indeed, as indicated in section 4.3.2, contact time was strongly associated with adherence and achievement of significant weight loss. This is likely due to individuals who attended more

study visits experiencing greater support and reinforcement of behaviour change strategies. This also further evidences that prior to commencing the intervention, consideration should be given to participants availability to attend study visits and further substantiates why irregular shift work may be a barrier to adherence for some individuals. Furthermore, it was reported consistently that support from other participants would have been beneficial, and provision of a summary of important outcomes from each visit (e.g., weight, BMI, blood pressure and HbA1c, where applicable) to take home would have further increased motivation to adhere.

There are some overlaps with the findings from this study and those conducted previously in the context of lifestyle behaviour change. For example, it has been reported that physicians play a crucial role in the advocacy of weight loss/ lifestyle behaviour interventions (Andersen et al., 1997, Navarro et al., 2007). This highlights the importance of clinicians being knowledgeable and appropriately trained to effectively refer patients to lifestyle behaviour change interventions and to provide positive reinforcement throughout the intervention period.

Prior to undertaking the VLCD, the majority of participants had attempted to lose weight and maintain weight loss with varying levels of success. However, clinical advice regarding weight loss in patients with NAFLD is often vague and unstructured and clinicians may benefit from training to improve the information they provide to patients and the way in which they do so to maximise behavioural change (Hallsworth et al., 2019, Alemany-Pagès et al., 2020). In general, awareness of NAFLD is low, even in patient populations at highest risk (Wieland et al., 2015), therefore it is likely that knowledge about the role of weight loss on prevention of progression of NAFLD is absent (Avery et al., 2017).

Facilitators to continued engagement and adherence widely encompassed several themes. For example, the use of meal replacements meant that patients did not have to prepare calorie-counted meals, and the small portions of vegetables gave participants the satisfaction and satiety associated with eating and preparing solid food. This was reported as helpful, particularly in the context of social and practical support outside of study visits was provided by family, friends and colleagues and was instrumental to maintaining long-term adherence. Data highlighted the importance of social support, wherever it may come from, as critically important to patients regardless of gender, age, amount of weight loss achieved, or length of

time since diagnosis. In order to facilitate integration of a VLCD into a clinical setting, it is important to identify at the beginning the source of social support that each patient will most positively respond to.

Achievement of early rapid weight loss was a significant facilitator to adherence. Participants reported feeling the effects of the VLCD early on and this motivated them to continue, while allowing them to take charge of their own health and regain control. Feedback on blood test and body composition results obtained during the study visits were a major factor influencing adherence highlighting the need for biofeedback.

When asked about ways in which the various components of the intervention could be improved to maximise engagement and adherence, several participants suggested that emotional and practical support from others undertaking the intervention would be valuable; a sentiment that was observed in a study of participants with T2DM (Rehackova et al., 2017). When considering the potential integration of a VLCD within a clinical setting, this could be a low-cost adjunct to the services provided by healthcare professionals. Additionally, a further suggestion from patients was to have an ongoing 'report card' where test results of interest could be written down to enable self-monitoring. Personalised feedback throughout interventions is a potentially useful strategy, particularly when closely accompanied by guidance on how to elicit further improvements (Polonsky and Fisher, 2015).

In general, the predominant themes reported from this qualitative study are largely supported by previous qualitative evaluations of VLCD studies in clinical populations including T2DM, obesity and polycystic ovarian syndrome (Rehackova et al., 2017, Love et al., 2016, Östberg et al., 2011). The salient motivators to uptake reported in our data have striking similarities to those reported in trials of VLCDs undertaken in people with T2DM. Specifically, the desire to achieve rapid weight loss was reported as a major incentive to uptake and engagement with the VLCD (Rehackova et al., 2017). In our study, alongside the desire to achieve significant weight loss for health purposes, a desire for rapid weight loss was demonstrated through patients wanting to dress in clothing that had once fitted them, become more confident, and subsequently feel better about their bodies as a consequence of the weight loss, as demonstrated in prior qualitative studies (Herriot et al., 2008, Vartanian et al., 2012).

It has been unanimously reported across T2DM, obese and polycystic ovarian populations undertaking a VLCD that a highly regimented intervention promoted adherence, i.e. that participants would not select the 'wrong' thing to eat. Similarly, the importance of support outside of a clinical setting, such as from the workplace, friends and family, has been reported in other qualitative evaluations of VLCDs (Rehackova et al., 2017, Love et al., 2016, Östberg et al., 2011). Our data also identified the importance of empowerment by participants taking charge of their own health. This has also been observed in the context of T2DM (Rehackova et al., 2017). This is critically important given that patient empowerment and consequential self-management is now at the forefront of healthcare, particularly within elements of the metabolic syndrome (Funnell and Anderson, 2004).

Overall, this data summarily reports that the VLCD is acceptable to many patients with NAFLD (Scragg et al., 2020), and has the potential to be a suitable treatment approach for some patients with NAFLD. In order to better understand the acceptability of the VLCD, it is important that this study is repeated in a larger, more diverse cohort in terms of ethnicity and age.

In order to successfully further evaluate the acceptability or potentially integrate into standard clinical care, our data provides important insight. For example, this data presents further evidence that clinician advocacy is effective in promoting adherence to a lifestyle intervention, and that a brief conversation between patients and their clinician, as a credible source of information, could encourage a patient with NAFLD to make some lifestyle changes. VLCDs, despite being cost effective and efficacious in eliciting significant weight loss, are reportedly underutilised by clinicians (Collins, 2003). Potentially, this underutilisation may be due to lack of confidence in 'prescribing' a VLCD, but as of yet not been investigated. However, the present data indicates that successful integration of a VLCD intervention into clinical care may be dependent on clinician training of the intervention.

Overall, social and practical support from friends, family members, work colleagues and members of the research time were identified as salient motivators to adherence. Regular study visits facilitated the building of rapport between members of the research team conducting the study visits and patients, which likely fed into patients feeling accounTable towards medical professionals. This was reported as an important motivator to adhere to the intervention. Similarly, it is important that patients have support from work colleagues or

employers, to facilitate time off work to be able to attend study visits, as well as allocating shifts accordingly to ensure patients are not overly fatigued. It is potentially important to many patients that prior to commencement of the VLCD that they discuss the implications and pragmatic elements with their work colleagues. It is important that, where applicable, family members are involved in the decision to undertake a VLCD. Indeed, within our cohort, we invited patients to bring family members to study visits, which patients reported to be another important form of social support.

Feedback from patients provides an essential tool for successfully further evaluating the potential acceptability of the VLCD. For example, it has been reported in our data, as well as in a T2DM cohort, that support off other people undertaking the intervention would be hugely valuable. Given that this could be provided at no extra cost to the running of the intervention, this is an avenue that should be further explored. Potentially the most important feedback to be derived from this study is the possible incompatibility of a VLCD with variable shift work. This was highlighted as a major barrier to adherence and therefore further evaluations of the intervention would be well placed to investigate how, and if, a VLCD could be adapted to suit these individuals.

4.4.1. Limitations

The primary limitation to this study is the lack of patient views reporting on barriers, facilitators and motivators to adherence and engagement throughout the weight maintenance phase. In order to better understand the strategies employed throughout the weight maintenance phase in order to minimise weight regain, interviews should have been undertaken at 9 months follow up. However, at the 9 month time point, the majority of patients did not have the flexibility around their working schedule to attend interview visits, on top of the regular study visits, and therefore data saturation would not have been achieved. Similarly, staffing arrangements within the research team and room hire arrangements within the Freeman Hospital meant that where patients were available for interview, a member of the research team or a spare room in which to host the interview was

not. Further studies should explore the utility of telephone interviews or surveys in order to add greater flexibility when exploring the qualitative data relating to the long-term acceptability of the study, such as throughout the weight maintenance phase.

Another limitation was the lack of referring clinicians input. Should this intervention be scaled up to be implemented in standard clinical care, or to inform larger studies in the future, the input of the referring clinicians would add greater understanding to the feasibility of recruiting to this intervention. However, in the present study, it was not possible to obtain a representative views of the referring clinicians, due to scheduling difficulties alongside their clinical practice. Furthermore, two of the referring clinicians were members of the research team and therefore may have presented biased feedback and may not have been representative of the other referring clinicians due to their personal interest in ensuring the intervention was fully recruited to. Potentially, future studies could explore the utility of clinician focus groups or explore other media to undertake interviews, such as telephones or surveys, to increase the likelihood of obtaining the relevant qualitative data in spite of scheduling difficulties.

The qualitative data revealed that involvement in a research study elicited significant motivation to adhere to the VLCD, which has previously been reported (Herriot et al., 2008). Furthermore, while this study intended to map onto usual clinical care, it is probable that the level of communication between patients and members of the research team acted as a facilitator to adherence and it would be difficult to replicate this intensity of communication routinely. The development of a relationship between members of the research team, particularly those conducting the interviews, may have reduced the likelihood that participants reported negatively on the intervention and associated practices, although a second member of the research team assisted in the conduct of interviews to help overcome this issue. Similarly, lapses in the adherence of the intervention may have been under reported due to self-preservation bias (Schlenker and Leary, 1982). Participants who did not complete the intervention were invited to be interviewed, but did not agree. Therefore, these data reflects only the opinions of those who completed the intervention. While only three participants did not complete the intervention, their views were not represented. Furthermore, of the original 45 people who were approached, 10 declined to take part (Chapter 3, Section 3.2.2), suggesting that the VLCD intervention is not acceptable to all

patients with advanced NAFLD. Further research should aim to formally report reasons for declining to take part, so that potentially the study could be adapted or invitation materials and conversations to increase uptake and potential acceptability. Finally, all participants within this study were Caucasian, and therefore the views of patients from other ethnic backgrounds not been explored. Future qualitative studies should aim to recruit those who refused to participate, or who did not find the VLCD intervention acceptable in order to develop strategies to overcome barriers.

4.5. Conclusions

The use of a VLCD to achieve significant weight-loss in adults with NAFLD is acceptable and feasible. Overall, patients found the intervention easier than anticipated but intensely rewarding. While barriers were identified, further research is required in a larger and more diverse group of individuals with NAFLD to explore motivators, facilitators and barriers in more detail to develop effective strategies to lifestyle behaviour change.

5. Objectively measured physical activity and sleep in an advanced NAFLD cohort

Abstract

Introduction: Non-alcoholic fatty liver disease (NAFLD) is associated with lower levels of physical activity (PA). We aimed to evaluate the differences in levels of PA and inactivity in patients with NAFLD and healthy controls using triaxial accelerometry, and establish if PA and sleep changed from baseline to post-VLCD and follow-up.

Methods: Twenty patients with advanced NAFLD were age- and gender-matched to healthy controls. Wrist-worn triaxial accelerometers assessed PA and inactivity over 24-hours for seven consecutive days.

PA, inactivity and sleep were objectively measured for 7 consecutive days at three time points- baseline, post-VLCD and follow up.

Results: Patients with NAFLD spent more time inactive (747 vs. 629 minutes/day; $p<0.05$) and less time engaging in PA of all intensities (light PA: 180 vs. 236 minutes/day, $p<0.05$; moderate PA: 28 vs. 120 minutes/day, $p<0.001$; and vigorous PA: 0 vs. 2 minutes/day, $p<0.01$) when compared to healthy controls. After controlling for BMI, moderate and unbouted moderate-vigorous PA were significantly lower in patients with NAFLD ($p<0.05$). No significant changes were observed in PA of any intensity, inactivity and sleep data between baseline, post-VLCD and follow up.

Conclusions: Targeted strategies to increase PA levels and decrease inactive time should be included as part of standard clinical care for patients with NAFLD. An 8-12 week VLCD programme did lead to any significant changes in PA, inactivity or sleep. Potentially, to assist in weight loss maintenance and counteract a reduction in skeletal muscle mass, patients should be encouraged to increase their level of PA after a VLCD.

5.1. Introduction

As previously discussed, non-alcoholic fatty liver disease (NAFLD) is the most common liver condition worldwide and is estimated to affect up to 33% of the Western population (Estes et al., 2018). Given that NAFLD is directly linked to chronic excess calorie consumption, lack of physical activity (PA)/exercise and being overweight/obese (Romero-Gómez et al., 2017), the increasing rates of obesity corroborate the projection of rising NAFLD incidence. Given that NAFLD is so strongly associated with lifestyle behaviours, and in the absence of approved pharmacological therapies, modification of an individual's lifestyle is the primary recommended treatment (NICE, 2016c). Weight loss is key in managing NAFLD (NICE, 2016c), but PA/exercise has been shown to have an independent effect on liver fat (Hallsworth et al., 2011, St. George et al., 2009, Johnson et al., 2009) and is a useful adjunct alongside dietary change to aide weight loss maintenance (Kistler et al., 2011, Krasnoff et al., 2008). Similarly, breaking up prolonged inactivity using approaches such as bouts of walking has been shown to be effective in attenuating postprandial glycaemia and inducing an energy deficit in high-risk obese and inactive individuals (Bailey and Locke, 2015, McCarthy et al., 2017).

Current PA guidelines for healthy adults recommend performing a minimum of 150 minutes of moderate PA or 75 minutes of vigorous PA per week (2020) however, up to 1/3 of people within the general population fail to achieve this (Cassidy et al., 2018, Brainard et al., 2020). Previous research has reported lower levels of PA in people with NAFLD when compared with healthy cohorts using self-report methods (Zelber-Sagi et al., 2008, Ryu et al., 2015). However, self-report methodologies are often less robust than objective methods (i.e. accelerometry) as they offer a less precise measurement (Shiroma et al., 2015). Similarly, self-report methodologies may include biased reporting of active and inactive behaviours and can subsequently reduce the magnitude of the observable relative risks in etiologic studies to an undetecTable value (Matthews et al., 2012). Furthermore, self-report may also be influenced by variations in mood, depression, anxiety, or cognitive ability (Rikli, 2000). Due to these limitations, daily step count became a popular objective alternative measure of physical activity, and found to be significantly lower in NAFLD compared to healthy controls (Newton et al., 2008). However, step count is largely limited in that it does not report on non-ambulatory modes of PA, nor does it always report on PA intensity (Ruth et al., 2006,

Chomistek et al., 2017). For a more accurate representation of an individuals' PA levels, 24-hour accelerometry is preferred over daily step count, as it allows for objective measurements of duration, frequency and amount of PA which can subsequently be defined as 'light', 'moderate' or 'vigorous' (Murphy, 2009, Hallsworth et al., 2015). 24-hour monitoring is beneficial as it allows for full inclusion of PA, sedentary time and sleep, thereby providing more detail about a patients' lifestyle. It also allows for time spent in inactive behaviors to be quantified, which in itself has been identified as an independent risk factor associated with obesity and increased cardiovascular risk (Ekelund et al., 2019, Vainshelboim et al., 2017). Inactivity, has been associated with reduced energy expenditure (Hallsworth et al., 2015) and an increased prevalence of NAFLD (Ryu et al., 2015).

Previous research has compared PA between NAFLD and healthy cohorts using biaxial accelerometry, where the NAFLD cohort was shown to engage in significantly less PA than the healthy controls (Hallsworth et al., 2015). However, triaxial accelerometry has been shown to be more accurate when gauging PA than biaxial (Shiroma et al., 2015, Howe et al., 2009). This is due to triaxial acceleromters detecting motion in three orthogonal planes (*V*, anterior-posterior (AP), and medial-lateral (ML)), rather than motion detected in two planes as reported by biaxial accelerometer (Plasqui and Westerterp, 2007). This is important as human motion is not limited to movements in the *V* plane, especially during activities of daily living. Gerber *et al* used triaxial accelerometry to evaluate activity in a NAFLD vs. non-NAFLD cohort (Gerber et al., 2012). This study (n=3056 participants), reported that average PA for people with NAFLD was approximately 29 minutes/day less than controls, with NAFLD patients spending less time participating in PA at any intensity.

Many people with NAFLD suffer from obstructive sleep apnoea (OSA) (Aron-Wisnewsky et al., 2016), and while studies have investigated the differences in sleep duration and efficiency between NAFLD populations and healthy controls, this has only been done using self-report questionnaires. Data so far indicates that people with NAFLD experience shorter sleep duration and poorer sleep quality (Marin-Alejandre et al., 2019, Imaizumi et al., 2015), but to date this has not been measured objectively.

It has previously been reported that increasing PA following a VLCD in obese individuals has been identified as an independent predictor for maintaining weight loss (Fogelholm et al., 1999, Leser et al., 2002). However, there are conflicting findings on the efficacy of increased

PA post-VLCD as a strategy for maintaining weight loss (Johansson et al., 2014). While changes in PA and sleep pre and post-VLCD haven't been reported in NAFLD patients before, no significant changes were reported in patients with T2DM pre and post-VLCD (Lean et al., 2019).

Similarly, changes in objectively measured sleep efficiency and duration haven't been investigated between baseline and post-VLCD in patients with NAFLD. However, improved sleep efficiency and longer duration after an 8 week VLCD have been reported in patients who are obese (BMI>30 kg/m^2) (Janus et al., 2020), and sleep quality has been consistently demonstrated to improve following significant weight loss, by either dietary or surgical methods in obese patients with OSA (Foster et al., 2009, Dixon et al., 2012).

Establishing key differences in PA, inactive behavior and sleep between people with NAFLD and healthy controls, and how levels differ from published guidelines/recommendations, is of paramount importance for tailoring PA advice, reducing time spent inactive and ensuring good quality sleep as a means of managing NAFLD in the clinical setting.

The purpose of this study was to objectively evaluate PA levels, inactivity and sleep using 24-hour tri-axial accelerometry in patients with clinically confirmed NAFLD and compare this to age- and gender-matched healthy controls. Furthermore, as an adjunct to the VLCD study (Chapter 3) we aimed to evaluate changes in PA levels and sleep data between pre-VLCD, immediately post-VLCD and at follow up. This study hypothesised that a NAFLD cohort would engage in less PA and more physical inactivity compared with age and gender matched healthy controls, and that people with advanced NAFLD experience poorer sleep quality than age and gender matched healthy controls. Furthermore, given that the VLCD intervention was purely dietary and not related to PA behaviours, this study hypothesised that patients with advanced NAFLD would not experience an increase in PA or decrease in physical inactivity. This study hypothesised that sleep quality (sleep efficiency) would increase between baseline and post-VLCD as a result of significant weight loss.

5.2. Methods

5.2.1. Recruitment and inclusion/exclusion criteria

As per the recruitment outlined in section 2.2.1, thirty patients with a clinical diagnosis of significant NAFLD were recruited from hepatology clinics within the Newcastle upon Tyne Hospitals NHS Foundation Trust (NuTH) from January-July 2019. Clinically significant NAFLD was defined as imaging evidence of steatosis plus an indeterminate or high NAFLD Fibrosis Score (≥ -1.455) or FIB-4 (≥1.3 if age <65; ≥ 2.0 if age ≥65) (Angulo et al., 2007, McPherson et al., 2017, McPherson et al., 2010), or histological evidence of NASH with fibrosis. Patients with compensated NASH cirrhosis (Child-Pugh score <7) were also eligible for recruitment. Other inclusion criteria specified age ≥18years, weight stability (+/-3%) since biopsy/non-invasive assessment of liver health and capacity to provide informed consent.

Patients were excluded if they had evidence of co-existent liver disease (e.g. autoimmune liver disease, viral hepatitis, alpha-1 anti-trypsin deficiency, haemochomatosis or Wilson's disease), decompensated NASH cirrhosis (Child Pugh score ≥ 7), excessive alcohol consumption (>21 units/week for males; >14 units/week for females), known cancer, myocardial infarction within six months and pregnant/considering pregnancy.

An age- and gender-matched healthy control group were recruited through advertisements at NuTH and Newcastle University, Newcastle upon Tyne, UK. Body weight (kg) and height (cm) were measured using an electronic stadiometer (SECA 799, SECA UK). BMI was subsequently calculated as body weight (kg)/ (height $(m)^2$).

As described in section 2.11, NAFLD patients as part of the VLCD study (Chapter 3) wore the wrist-worn accelerometer for seven consecutive days at three time points throughout the study period; baseline, post VLCD and at follow up.

The study protocol was approved by North East-Newcastle & North Tyneside 1 Research Ethics Committee (REC reference: 18/NE/0179) (ISRCTN Register: ISRCTN85177264) and Newcastle University Research Office (reference: 2901/2017). All participants provided written informed consent. The funders played no role in the design, conduct, or reporting of this study. All procedures were performed in accordance with the Declaration of Helsinki.

5.2.2. Accelerometer data analysis

Participants in both groups were instructed to wear the GENEActiv tri-axial accelerometer (ActivInsights Ltd, United Kingdom) continuously on either wrist for seven consecutive days. Accelerometers were returned by post and the data downloaded and stored. Accelerometer data was only included for further analysis if the monitor wear time included at least three of the seven monitored days and at least one weekend day.

Raw accelerometer data was processed in R (www.cran.r-project.org) using R-package GGIR (Version 2.0-0) (Van Hees et al., 2013, Migueles et al., 2019, RCore, 2016) . Calibration error of the signals were inspected and corrected as described previously (Van Hees et al., 2014). The inclusion of at least 16 hours of valid data for inclusion in the analysis has been described elsewhere (Charman et al., 2016). The average magnitude of wrist acceleration per 5 second epoch was calculated with metric ENMO (1 m*g* = 0.001 x gravitational acceleration) as previously described (Van Hees et al., 2013). Monitor non-wear has been described previously (Van Hees et al., 2013) and was replaced by the average accelerometer data on similar time points on different days of the measurement (Van Hees et al., 2014, Sabia et al., 2014). The imputation procedure has been described elsewhere (Charman et al., 2016). Time spent in the following acceleration thresholds were calculated: inactivity (<40 mg cut-off) light PA (40-100 mg cut-off); moderate PA (100-400 mg cut-off), vigorous PA (>400 mg cut-off), moderate-vigorous PA (MVPA) (≥100 mg cut-off) (Hildebrand et al., 2014, Cassidy et al., 2018). Bouts of MVPA are identified as all 1 or 5 minute time windows that start with a 5 second epoch value equal or higher than 100 mg and for which 80% of subsequent 5 s epoch values are equal to or higher than the 100 mg threshold (Hildebrand et al., 2014). Estimated total sleep duration (minutes) and sleep efficiency (%) based on absence of change in arm angle greater than 5 degrees for a time period of 5 minutes or longer has previously been described (Van Hees et al., 2015).

5.2.3. Statistical Analyses

All statistical analyses were performed using IBM SPSS version 24 (IBM, Armonk NY, USA). Statistical significance was set at $p<0.05$. Data were assessed for normality and outliers using Shapiro-Wilk and boxplots. Non-normal data was either log-10 or square-root transformed, or assessed using a Mann-Whitney U test. Differences between groups were assessed using

an independent students T-test. The mean BMI was significantly higher in the healthy cohort when compared with the NAFLD cohort and BMI has previously been associated with lower levels of PA (Petersen et al., 2004). Therefore, an ANCOVA controlling for BMI was undertaken to establish if the significant differences between the groups persisted when adjusted for BMI. Data is presented throughout as median (range) unless stated otherwise.

5.3. Results

5.3.1. NAFLD cohort vs. healthy cohort

5.3.1.1. Characteristics of subjects and available data

The patients within the NAFLD cohort who had analysable data at baseline (Figure 2.1) were age- and gender-matched to healthy controls (see Table 5.1). The median BMI for NAFLD and healthy controls were significantly different (37.9 vs 24.0 kg/m^2; $p<0.001$). Given the nature of accelerometery data collection and relative likelihood of technical failure, not all data was able to be analysed. Indeed, within the NAFLD cohort, technical failure was caused by data being stored in the incorrect format for analysis (Figure 5.1). No technical failures were observed in the healthy cohort. Furthermore, in order to maximise accuracy, datasets were not analysed if they did not meet the threshold for minimum wear time (as described in section 5.2.2). As indicated in Figure 5.1, after accounting missing data, twenty patients from the NAFLD cohort had analysable data which was then subsequently age and gender matched to twenty healthy volunteers.

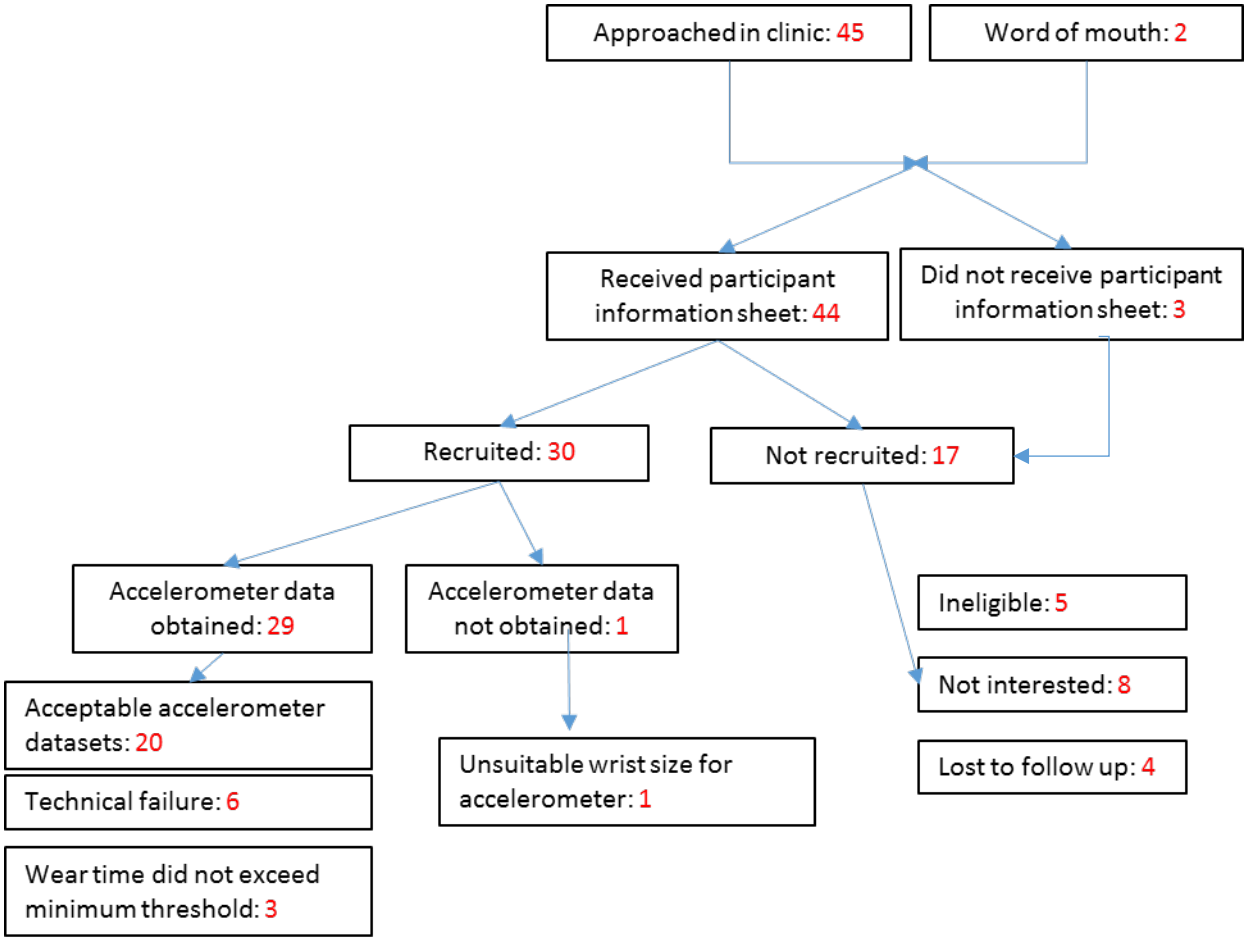


Figure 5.1. CONSORT diagram to show recruitment and participant flow of NAFLD arm

5.3.1.2. Wear time

Median wear time (days) for both groups was 6 days (Table 5.1), with a range of 3-7 days being achieved. Of the NAFLD cohort, 15% of subjects wore the accelerometer for 7 days compared to 40% within the healthy controls. Overall, general adherence was high -within the days that data was captured, median wear time (minutes) was 1438 for both cohorts, where 1440 would represent the whole 24 hours.

5.3.1.3. Inactivity and activity levels

Significant differences were observed in time spent engaged in inactive behaviours, and different PA intensities between the groups. Inactive behaviours (shown in Table 5.1), were significantly higher in the NAFLD cohort compared to the healthy cohort (747 vs. 623 minutes/day; p=0.026), equating to a difference of approximately 2 hours per day. Time spent engaged in 'light' PA was significantly lower in the NAFLD cohort than the healthy cohort (180 vs. 236 minutes/day; p=0.043), with a similar trend shown in moderate PA (28 vs. 120

minutes/day; p<0.001) and vigorous PA (0 vs. 1.9 minutes/day; p=0.003) respectively. Time spent in MVPA combined was significantly lower in patients with NAFLD compared to healthy controls (29 vs. 127 minutes/day; p<0.001). Time spent engaging in bouts of MVPA for over 5 minutes were significantly higher in the healthy controls (9 vs. 28 minutes/day; p<0.001). Similarly, time spent engaging in bouts of MVPA for over 10 minutes were significantly higher within the healthy controls (5 vs. 28 minutes/day; p<0.001), as shown in Figure 5.2. When adjusted for BMI, moderate and unbouted moderate-vigorous PA remained significantly different between healthy controls and the NAFLD cohort.

Overall, results indicate that the NAFLD cohort spent less time engaged in PA of any intensity when compared to their healthy controls, shown in Figures 5.2A-D.

5.3.1.4. Sleep data

Sleep data was not statistically significantly different between the NAFLD and healthy cohorts (Table 5.1). However, sleep duration was marginally lower in the NAFLD cohort (360 vs. 388 minutes, p=0.201) and sleep efficiency less (87 vs. 90%, p=0.072). Within the NAFLD cohort, 2 patients had obstructive sleep apnoea (OSA). These patients both reported sleep duration (299 and 306 minutes) in the lowest quartile of the NAFLD cohort and sleep efficiency (83 and 87%) were both lower than median value presented below.

Table 5.1. Characteristics and objectively measured inactive behaviour, PA levels, sleep duration and efficiency of NAFLD and healthy cohorts

Characteristics	**NAFLD**	**Healthy**	**P value**	**Adjusted P value**
Mean Age (years)	58.4 ± 12.5	56.9 ± 11	0.502	
BMI (kg/m^2)	37.9 (30.2-62.3)	24.0 (20.0-37.7)	<0.001***	
Gender (m/f)	10/10	10/10		
Variables				
Wear time (days)	6 (3-7)	6 (3-7)	0.966	

Wear time (minutes)	1438 (1190-1440)	1438 (1140-1440)	0.049*	
Sleep duration (minutes)	360 (186-467)	388 (233-506)	0.201	
Sleep efficiency (%)	87 (70-93)	90 (65-95)	0.072	
Time inactive (minutes)	747 (514-996)	623 (249-770)	0.026*	0.325
Time light (minutes)	180 (62-341)	236 (122-376)	0.043*	0.758
Time moderate (minutes)	28 (2-175)	120 (27-199)	<0.001***	0.028*
Time vigorous (minutes)	0 (0-4.8)	2 (0-37)	0.003**	0.376
Time moderate-vigorous (minutes)	28 (2-176)	127 (27-205)	<0.001***	0.025*
Time MVPA bouts >5 minutes (mean)	9 ± 15	28 ± 15	<0.001***	0.117
Time MVPA bouts>10 minutes (mean)	5 ± 11	28 ± 24	<0.001***	0.065

*denotes statistical significance p<0.05

**denotes statistical significance p<0.01

***denotes statistical significance p<0.001

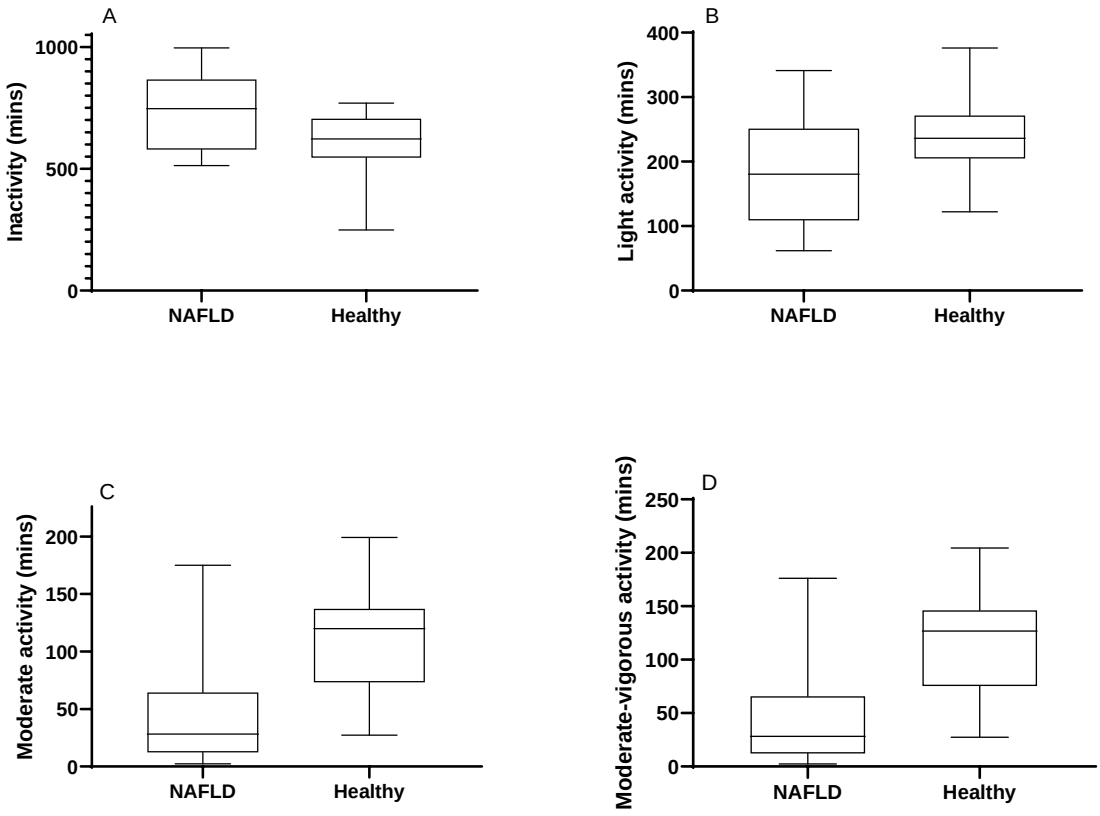


Figure 5.2. A breakdown of time spent engaging in inactive behaviour and the different intensities of PA. A) Time spent engaged in inactive activity. B) Time spent engaged in light activity. C) Time spent engaging in moderate activity. D) Time spent engaged in moderate-vigorous activity. Vigorous-only boxplot was excluded due to data being too low to be graphically represented.

5.3.1.5. Correlations between BMI and physical activity

Within the NAFLD cohort, BMI was significantly positively correlated with time spent engaged in inactive behaviour (r=0.523, p=0.018). Similarly, BMI was also significantly negatively correlated with time spent in moderate and moderate-vigorous activity (r=0.477, p=0.034 and r=0.474,p=0.035, respectively). Within the healthy cohort, BMI was significantly positively correlated with time spent engaged in inactive behaviour (r=0,612, p=0.004).

Table 5.2. The correlation between BMI and PA intensity levels in the NAFLD cohort and healthy controls.

PA intensity	NAFLD	Healthy
Inactivity	R=0.523, p=0.018*	R=0.612, p=0.004**
Light	R=-0.382, 0.097	R=-0.224, p=0.342
Moderate	R=-0.477, 0.034*	R=0.020, p=0.935
Vigorous	R=-0.199, p=0.400	R=-0.130, p=0.586
Moderate-Vigorous	R=-0.474, p=0.035*	R=-0.018, p=0.940

*denotes statistical significance p<0.05

**denotes statistical significance p<0.01

***denotes statistical significance p<0.001

5.3.2. Changes in PA levels and sleep following a VLCD

5.3.2.1. Available data

Taking into account patients dropping out from the VLCD study, technical failures and not all patients achieving the threshold for minimum wear time, complete datasets were available as indicated in Table 5.3. Overall, there were 12 patients with data analysable over three time points. The six technical failures related to the data from the GeneActiv being stored in the incorrect format for analysis.

Table 5.3. Total analysable datasets for each time points

Time point	Drop out	Technical failure	Less than minimum wear time	Total analysable datasets
Pre VLCD (n=30)	0	6	3	20
Post VLCD (n=27)	3	3	3	21

Follow up (n=20)	10	1	2	17

5.3.2.2. Activity levels

Across baseline, post-VLCD and follow up, no significant differences were observed in activity levels of all intensities (see Table 5.4 and Figure 5.3).

Table 5.4. Activity levels at each time point throughout the intervention

Variable	**Baseline (n=12)**	**Post VLCD (n=12)**	**Follow up (n=12)**	**P value**
Inactivity (minutes)	710±55	706±104	774±211	0.305
Light (minutes)	177±74	208±80	180±80	0.207
Moderate (minutes)	37±28	62±61	67±70	0.246
Vigorous (minutes)	0.6±1.5	1.0±2.2	0.2±0.4	0.713
Time moderate-vigorous (minutes)	37±29	63±63	67±70	0.863
Time MVPA bouts >5 minutes (mean)	5±4	13±14	14±21	0.943
Time MVPA bouts >10 minutes (mean)	2±5	10±18	13±23	0.363

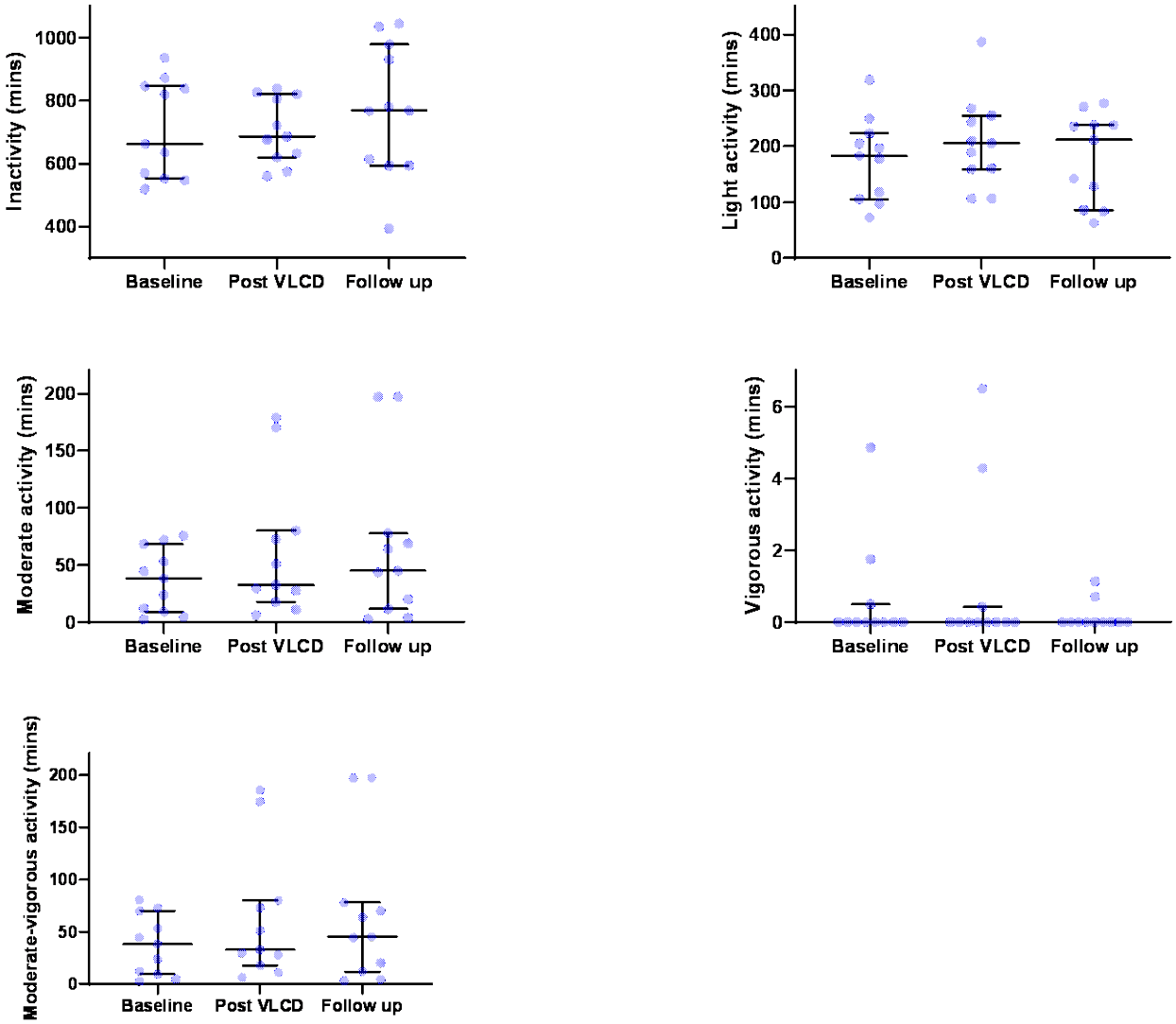


Figure 5.3. Changes in A) inactivity, B) light activity, C) moderate activity, D) vigorous activity and E) moderate-vigorous activity between baseline, post-VLCD and follow up.

5.3.2.3. Sleep data

There were no significant changes in sleep duration or sleep efficiency between baseline, post VLCD and follow up (see Table 5.5 and Figure 5.4).

Table 5.5. Sleep duration and efficiency at each time point throughout the intervention

Variable	Baseline (n=12)	Post VLCD (n=12)	Follow up (n=12)	P value
Sleep duration (minutes)	353±75	356±93	322±80	0.303
Sleep efficiency (%)	85±7	83±12	86±8	0.969

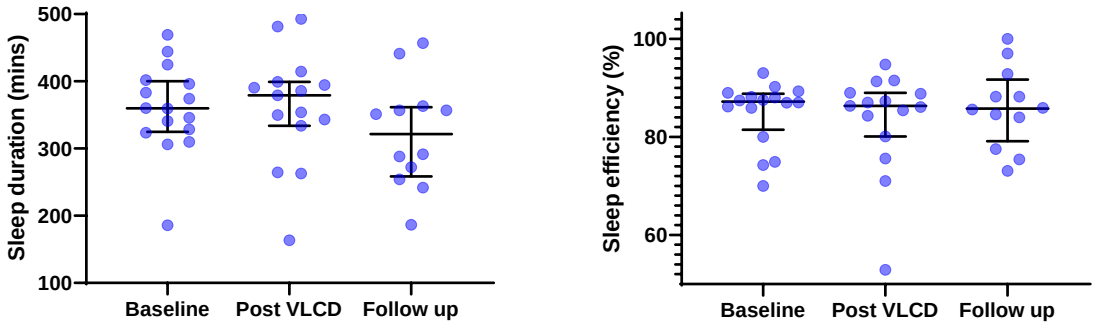


Figure 5.4. Changes in A) sleep duration and B) sleep efficiency between baseline, post-VLCD and follow up.

5.4. Discussion

5.4.1. NAFLD vs. healthy controls

This is the first study to compare activity levels between an advanced NAFLD cohort and age- and gender- matched healthy controls using tri-axial accelerometery over 24 hours to measure sleep, PA and inactive behaviour. The findings reveal individuals with NAFLD have 1) lower levels of PA across all intensity domains, 2) higher levels of inactive behaviour and 3) a trend towards poorer sleep quality and shorter sleep duration, compared to healthy counterparts. When controlling for BMI, only MVPA remained significantly lower, suggesting overweight/obesity is a key contributor to a reduction in overall PA levels and an increase in inactivity behaviour in people with NAFLD.

5.4.1.1. Physical activity

While a number of studies have described differences in PA levels between healthy populations and those with NAFLD, to date, the data presented is the first of its kind to be specifically compared between age- and gender-matched groups using tri-axial accelerometry. This study describes a clear differentiation of activity patterns between the two cohorts.

Historically, methods used to ascertain PA levels have primarily used self-report (Van Hees et al., 2014), where it was observed that individuals with NAFLD partake in less 'all intensity' activity, than healthy individuals (Zelber-Sagi et al., 2008, Ryu et al., 2015). While this NAFLD cohort had been formally diagnosed using imaging techniques, this study is largely limited by the methodology employed to ascertain PA levels. Another study evaluated the difference in activity between a large NAFLD population and compared this to a non-NAFLD population (Gerber et al., 2012). Significant differences were observed in PA between large populations. Individuals with NAFLD were in the lowest quartile of both average and moderate-vigorous PA ($P < 0.01$). However, patients were classified as having NAFLD incidentally following a non-invasive scoring system- the Fatty Liver Index (Bedogni et al., 2006). While the FLI is easy and widely applicable to clinical care, it is possible that it may classify individuals who are simply overweight as having NAFLD, as BMI is the main contributing factor to the FLI score. Furthermore, only 10h/day over seven consecutive days of activity data was analysed,

increasing the likelihood that activity levels could be significantly over- or under- estimated. Similarly, the healthy comparator arm was not age- or gender-matched. Therefore, the current data presents a novel viewpoint of differences in PA between a NAFLD cohort and healthy individual; the inclusion of patients who have been clinically diagnosed with NAFLD and the use of objective methodologies for measuring inactivity, PA and sleep.

The presented data adds further valuable information on the disparity of PA levels between people with NAFLD and healthy controls. Given that NAFLD is directly associated with excess caloric consumption and lower levels of PA and exercise, this study further supports the recommendations that all patients with NAFLD should be encouraged to increase their PA levels and reduce sedentary time where possible. One of the barriers to uptake of PA and exercise within individuals with NAFLD has been identified as a lack of confidence due to fear of falling, despite understanding the benefits of exercise (Frith et al., 2010). Therefore, tailored PA/exercise interventions are key to provide reassurance and guidance for people with NAFLD to facilitate safe uptake of PA.

There exists a complex paradigm between disease burden and the feasibility of engaging in regular PA, as many individuals with clinical diagnoses of NAFLD and/or obesity often perceive barriers to PA that healthy individuals may not, such as a lack of confidence or a fear of falling, and subsequently this perpetuates an ongoing cycle of insufficient PA and worsening disease burden (Frith et al., 2010). It is therefore of critical importance that individuals with NAFLD are educated on the positive effects that increasing PA/reducing inactivity could have for them, while being provided with personalised recommendations regarding PA that could be suitable for them.

5.4.1.2. Inactivity

Previous studies have described greater levels of inactivity in NAFLD cohorts compared to healthy populations (Ryu et al., 2015, Hallsworth et al., 2015), usually intrinsically coupled with lower levels of 'all-intensity' activity, as aforementioned. The data presented is the first of its kind to identify greater levels of inactivity using tri-axial accelerometery.

Increased time in physical inactivity and sedentary behaviours has been associated with obesity and increased body weight (Martínez-González et al., 1999). Given that NAFLD is

strongly associated with increased caloric intake, reduced time spent engaging in PA/exercise and inactive behaviour (Trovato et al., 2013, Centis et al., 2013), this presents an area within individuals lifestyle that could be targeted therapeutically. For example, this could provide an opportunity to encourage those with NAFLD to break up time spent being inactive using approaches such as bouts of walking or seated arm ergometry, as has been shown as effective in attenuating postprandial glycaemia and inducing an energy deficit in high-risk obese and sedentary individuals (Bailey and Locke, 2015, McCarthy et al., 2017). Given the prominent crossover of caloric surplus and dysregulated glycaemia in NAFLD populations, this could be an important means of managing and combatting some of the clinical burden induced by NAFLD.

5.4.1.3. Sleep

Despite no statistically significant difference in sleep, there is a trend towards reduced duration and efficiency/quality in those with NAFLD compared with healthy controls. It has previously been reported that NAFLD populations experience shorter sleep duration and poorer sleep efficiency than healthy controls (Marin-Alejandre et al., 2019). While the data presented reports different observations, it is important to acknowledge differing methods of data collection namely objective vs. self-report questionnaire. Imaizumi *et al* assessed sleep duration by questionnaire and subsequently grouped sleep duration into time brackets and assessed for correlation (Imaizumi et al., 2015). Sleep duration of <6 hours was associated with prevalence of NAFLD in women. Similarly, another study used the Pittsburgh Sleep Quality Index to assess the differences in sleep characteristics between normal weight non-NAFLD individuals and obese NAFLD individuals (Marin-Alejandre et al., 2019). In this NAFLD cohort, a higher prevalence of short sleep duration and poor sleep quality were found and sleep quality predicted up to 20% of the variability of liver stiffness, after adjusting for potential confounders. However, it is likely that BMI and body weight may have played an important role in these findings. It is important to consider that the presented data did not depict any significant differences in sleep, however, as aforementioned, other studies have.

While primary treatments for NAFLD target weight loss through diet and increased exercise/ PA, the presented and existing data regarding sleep dysregulation in patients with NAFLD provide an argument for the assessment of sleep in patients with NAFLD. This could allow for

subsequent interventions or referral pathways to be explored as a means of improving sleep within NAFLD populations. The prevalence of sleep apnoea in NAFLD populations has been reported as approximately 46% (Singh et al., 2005) and, as such, it is suggested that they share interlinked pathophysiology (Aron-Wisnewsky et al., 2016). Therefore, it is important that healthcare providers assess and treat sleep dysregulation as a key compartment of NAFLD.

5.4.1.4. Controlling for BMI

When adjusted for BMI, the differences in PA levels between the NAFLD and healthy controls are largely diminished, indicating that body weight is likely a key influencer of PA. However, the data still highlights significant differences in levels of moderate PA between groups. Similarly, there is a trend towards significance in MVPA bouts of greater than 10 minutes between groups.

Other studies that have evaluated PA in NAFLD and healthy cohorts (Hallsworth et al., 2015, Gerber et al., 2012) have not controlled for body weight, and observations have been made that a higher BMI is associated with lower levels of PA in NAFLD patients (Hallsworth et al., 2015). It is challenging to appropriately age, gender and BMI match a healthy population to a NAFLD population, as it is difficult to recruit individuals with a high BMI who are otherwise healthy. This dataset is novel in identifying varying levels of PA between NAFLD and healthy groups, in spite of the challenges that BMI matching presents.

Obesity without NAFLD is strongly associated with a reduction in PA levels across all intensities (Hansen et al., 2013, Van Dyck et al., 2015), indicating a similar pattern to our data. It is probable, and evidenced in the data presented, that BMI confers a great influence over levels of PA. The relationship between BMI and PA is a complex paradigm. It is unfeasible to dissect obesity from NAFLD as they are physiologically linked as part of the disease process. While the 'lean NAFLD' phenotype is a widely recognised characteristic of the NAFLD spectrum (Chen and Yan, 2006) it is important to consider that obesity is an inherent aspect of NAFLD for the majority of patients, and is a major shareholder in the clinical burden of NAFLD. It is therefore of utmost importance to account for this when considering potential therapeutic interventions for those with NAFLD.

5.4.2. Physical activity, inactivity and sleep throughout a VLCD intervention

5.4.2.1. Physical Activity

There were no significant changes in PA of all intensities between baseline, post VLCD and follow up. However, throughout the VLCD, given the significant caloric deficit, patients were not encouraged to partake in any extra PA and therefore significant changes between baseline and post-VLCD were not expected.

Previously, Lean *et al.* have evaluated the changes in PA at baseline and 12 months follow-up post-VLCD in patients with T2DM (Lean et al., 2018). At baseline, mean levels of light, moderate and vigorous activity (minutes/day) were 118, 51 and 1, respectively. At follow up, mean levels of light, moderate and vigorous activity (minutes/day) were 118, 51 and 1, respectively. PA did not significantly change between baseline and follow up for light activity (p=0.618), moderate activity (p=0.811) and vigorous activity (p=0.840). This pattern of change is consistent with the changes presented in the current dataset, and the levels of moderate and vigorous PA were similar between studies, while light intensity was higher within the NAFLD cohort. Similarly, objectively measured PA in an obese cohort showed no significant changes between baseline and at follow up after bariatric surgery (Afshar et al., 2017), indicating that patients do not necessarily make changes to their lifestyle despite achieving significant weight loss. However, changes in PA following significant weight loss have not previously been reported in patients with NAFLD.

Given the importance of caloric surplus and insufficient PA in the progression of NAFLD, it is of great importance that following significant weight loss, patients adapt their lifestyle behaviours to prevent recurrence of indicators of NAFLD. This is of particular importance as increased PA has been strongly associated with reduced weight gain at 3 years after a VLCD (Leser et al., 2002, Fogelholm et al., 1999). This presents an opportune period in which patients should be encouraged to increase PA to maintain achieved weight loss.

5.4.2.2. Inactivity

In the present study, inactivity did not significantly change between any time points. Inactivity has been associated with increased insulin resistance and an increased risk of T2DM, NAFLD and metabolic syndrome, particularly in obese/ overweight adults (Amati et al., 2009, Arias-

Loste et al., 2014, Mohan et al., 2005, Rector and Thyfault, 2011). Therefore, alongside weight loss, it is important that physical inactivity is reduced.

Previous studies have investigated the changes in time spent engaged in physical inactivity following significant weight loss, achieved using dietary interventions, in overweight/ obese patients and have reported no significant changes in time spent being inactive (Weinsier et al., 2000, Bartholdy et al., 2020). This suggests inactivity is not frequently addressed alongside other lifestyle changes that have been used to elicit weight loss, despite the concurrent health benefits that have been reported, and that patients do not alter activity patterns in response to weight loss.

5.4.2.3. Sleep

Our data reported no significant changes in sleep duration and sleep efficiency. Similarly, the DiRECT study reported no changes in the sleep efficiency (73 vs. 72, p=0.5066) or duration (421 vs. 443, p=0.4522) following a VLCD in T2DM (Lean et al., 2018). Within our NAFLD cohort, sleep duration was much lower while efficiency was higher than the T2MD cohort of the DiRECT study. Despite a relatively large number of patients within the NAFLD cohort having obstructive sleep apnea (OSA), of which weight loss is recommended as a management strategy (Chirinos et al., 2014), improvements in this specific subgroup were not vast enough to significantly improve mean sleep efficiency and duration. Significant weight loss, induced by both VLCDs and gastric bypass surgery, have been shown to improve OSA and subsequent sleep quality in obese patients (Suratt et al., 1992, Dixon et al., 2012, Kansanen et al., 1998), with further research suggesting that those who lose over 10 kg experience the greatest improvements in OSA in overweight patients with T2DM (Foster et al., 2009). However, this has not been investigated in NAFLD- specific cohorts and therefore the presented data is novel in evaluating this.

Improvements in sleep duration and efficiency are also associated with improvements in anxiety, depression and insulin resistance which are commonly associated with NAFLD (Van Cauter, 2011, Gregory et al., 2011). Poor sleep efficiency and reduced sleep duration have been associated with obesity and insulin resistance (Reutrakul and Van Cauter, 2018). Concurrently, short sleep duration and OSA have been consistently shown to increase hunger, appetite and food intake, with the increase in caloric intake in excess of the energy requirements of extended wakefulness (Reutrakul and Van Cauter, 2018, Murphy et al.,

2017). Therefore, the utilisation of significant weight loss to improve elements of NAFLD could potentially be achieved through both the reduction of hepatic adiposity and the improvement of sleep duration and efficiency to improve insulin resistance, appetite, satiety and glucose tolerance.

While significant changes in sleep duration and efficiency were not observed within our NAFLD cohort, this could be due to not enough patients losing a significantly large enough amount of weight to positively affect sleep or a small sample size of analyzable data across all time points. However, this provides an area where further research in larger cohorts would be of significant clinical importance to patients with NAFLD.

5.4.3. Strengths

All individuals within our NAFLD cohort were clinically diagnosed using biopsy, imaging techniques or histological parameters, and were under the care of consultant hepatologists, allowing for a definitive diagnosis of advanced NAFLD.

Age matching is of particular importance, as NAFLD is most prevalent in the 5th and 6th decades of life (Lazo et al., 2013), therefore comparator arms should aim to match this. Previous studies have reported significant differences in both age and gender between groups, with NAFLD arms generally being older and having a higher proportion of males (Gerber et al., 2012, Van Hees et al., 2014). This could potentially add selection bias to reported measures of PA between groups as previous research has indicates that PA is generally higher amongst adult males compared to females (Azevedo et al., 2007, Monteiro et al., 2003, Martinez-Gonzalez et al., 2001) and is lower in older adults compared to younger adults (Sallis and Saelens, 2000). Our data was gender-matched, with comparator paired ages matched within ±3 years of one another.

This study benefited from the use of tri-axial accelerometers, which have been shown to be more accurate that uniaxial and biaxial accelerometers when estimating PA (Howe et al., 2009, Shiroma et al., 2015). Tri-axial accelerometers were wrist worn for with 24 hour monitoring, improving compliance and reducing non-wear time. This is beneficial as discrepancies have been observed where both objective and self-report methods have been used in the same obese cohort as part of the same study (Afshar et al., 2017). Importantly,

this data is unique as it is the first study to evaluate sleep and PA following a VLCD in a NAFLD cohort.

5.4.4. Limitations

Our study did have some limitations, the most major pertaining to our small sample size. As is the nature with accelerometer data, not all patients wore the accelerometers for the required length of time. We chose a minimum of 3 days wear time to ensure that the data analysed was more likely to be representative of the individuals' day to day activities (Doherty et al., 2017). The main reason for not obtaining accelerometer data was technical failure. In most incidences where this happened, data was not stored in the correct format to be subsequently analysed.

Another limitation with this dataset is the differing average BMI between groups. While we aimed to, and successfully did, age- and gender-match our NAFLD cohort to healthy controls, it was not possible to match BMI. Given the nature of NAFLD and its strong association with obesity, it would difficult to find individuals with a BMI classified as 'obese' but who were otherwise healthy. However, to account for this, the datasets were analysed with adjustments for differing BMI between groups as previously described.

The healthy cohort presented were predominately selected from university/hospital staff, their families or post-graduate students, likely leading to a largely homogenous group of individuals who were highly educated and not of a lower economic status. Studies have reported on the effect of socioeconomic status and health outcomes, with a positive correlation found between lower socioeconomic status and poorer health outcomes (Pickett and Pearl, 2001). Further research in this area would benefit from including participants from a wider range socioeconomic backgrounds, to allow for a truer representation of a standard 'healthy' cohort within the UK.

5.5. Conclusions

This study aimed to evaluate and compare PA levels between a NAFLD and a healthy cohort. The results from this study show a significantly different pattern of PA between the groups, with the NAFLD cohort engaging in lower amounts of 'all-intensity' PA compared with the NAFLD cohort, as well as spending more time engaged in inactive behaviours. No significant differences were observed in sleep duration or efficiency. Overall, this presents an

opportunity where PA could be targeted therapeutically in order to improve NAFLD and reduce the risk of disease progression. This also provides further evidence of the importance of targeting PA with NAFLD populations, and raising awareness within both patient and healthcare provider populations that increasing PA could be a powerful tool in combatting NAFLD.

6. Assessing the needs of patients awaiting liver transplantation: Development of a targeted exercise intervention

Abstract

Background: The prevalence of liver disease, and subsequently the demand for liver transplantation is increasing annually. Lifestyle behaviours and measures of functional capacity have previously been identified as major indicators of mortality post-transplant and as such are now assessed as part of the suitability for transplant to determine whether a patient is listed or not. This study aimed to (i) undertake and subsequently utilise a retrospective cohort analysis of all patients assessed for suitability for liver transplantation and; (ii) conduct focus group discussions with patients and healthcare professionals to inform the development of an exercise intervention for patients awaiting liver transplantation.

Methods: Data were collected from all patients being assessed for liver transplantation between January 2014- May 2018 at one regional transplant centre in the UK to conduct a cohort analysis. Focus group discussions were undertaken with a purposively sampled group of patients and health care professionals to inform the development of the exercise intervention.

Results: Of 332 patients assessed, 47% were listed for transplant. The need for lifestyle modification was one of the primary reasons for patients not being listed, and measures of functional capacity were significantly lower in those who were considered to be suitable for liver transplantation relative to population normative values. Patients reported that they would be keen to take part in an exercise intervention while on the waiting list if it improved their outcomes, and that information about the benefits of exercise on liver transplantation outcomes would act as a motivator to take part. Safety concerns were reported to be a barrier to uptake and adherence.

Conclusion: This research has identified a cohort of patients who would benefit from lifestyle optimisation prior to liver transplantation. Patients who are on the waiting list, or have undergone a liver transplantation would be motivated to partake in an exercise intervention prior to the liver transplantation.

6.1. Outline of Chapter

This Chapter reports on the development of an exercise intervention targeted towards patients who are on the active waiting list for liver transplantation and is divided into two phases: Phase one involved a retrospective cohort analysis that was undertaken to quantify and define the proposed target population. Phase two involved development of the exercise intervention, informed by findings from the qualitative focus group discussions with patients and healthcare professionals.

6.2. Phase one: retrospective cohort analysis

6.2.1. Introduction

Liver disease is the fifth most common cause of death in the UK (Murray et al., 2013) with increasing prevalence in recent years (Pimpin et al., 2018). Liver transplantation is a successful treatment for end-stage liver disease with current one and five year survivals in excess of 90% and 70%, respectively (NHS, 2019). Over the last decade, the demand for liver transplantation in the UK has steadily increased (NHS, 2017, NHS, 2019)) and the average time on the waiting list for liver transplant is 99 days (NHS, 2019). This provides an opportunity to support patients on the waiting list to make lifestyle behaviour changes to optimise post-surgical outcomes.

It is well recognised that liver transplantation is associated with the onset of a number of conditions that increase risk of early mortality, including new onset diabetes, hypertension and dyslipidaemia (Benhamou and Penfornis, 2002, Gisbert et al., 1997). These conditions have the potential to be prevented or improved by behavioural intervention targeting health and lifestyle behaviours including physical activity, exercise, diet, smoking and medication adherence (De Luca et al., 2015). Lifestyle factors are central to both survival on the waiting list and long-term post-transplant survival. Therefore it is important to support patients to make positive and sustainable lifestyle behaviour changes. However, this represents a complex medical challenge because rarely are clinical teams trained to target lifestyle behaviour change in a meaningful and personalised way.

Guidelines for optimising pre-transplant health now incorporate recommendations to target smoking cessation, alcohol reduction/cessation, dietary modification and physical activity levels (Burra et al., 2016, Murray and Carithers Jr, 2005). Currently there are no universally

agreed approaches to targeting these lifestyle behaviours in clinical practice. Research has shown that a combination of diet and exercise is the most effective approach for weight loss (Marchesini et al., 2016) and growing evidence highlights that higher cardiorespiratory fitness prior to surgery, improves post-transplantation outcomes (Prentis et al., 2012). Furthermore, frailty has been shown to be indicative of waitlist mortality (Lai et al., 2014). However, there is limited guidance to provide specific recommendations on the type, amount and intensity of exercise that would be most beneficial for safely improving or maintaining fitness in pre-liver transplant cohorts. Few studies have evaluated the reasons for ineligibility, and proportion of patients ineligible for liver transplantation; and of those that have assessed reasons for ineligibility, functional status and lifestyle behaviours have not been reported (Kemmer et al., 2011, Arya et al., 2013). A single-centre Canadian study reported that 49% of patients assessed were ineligible for transplant (Arya et al., 2013). Reasons for this included psychosocial contraindications, and being referred to assessment for suitability too early (ie. needing further investigations). Kemmer *et al.* specifically assessed a cohort of patients who were ineligible for transplantation, however, functional capacity and frailty were not measured (Kemmer et al., 2011). A greater understanding of patients who are ineligible for liver transplant could serve to establish an intervention pathway for this cohort, with the goal of improving candidacy for transplantation.

The primary aim of the cohort analysis was to characterise the lifestyle behaviours and functional capacity of patients being assessed for liver transplantation. The findings of the analysis would inform the development of an exercise intervention for patients awaiting liver transplant. The hypothesis of this cohort analyses was that people being assessed for suitability to receive a liver transplant would have poor functional capacity, as described using measures of physical fitness determined by a CPET. Similarly, it was therefore hypothesised that people on the active waiting list for liver transplant would have low levels of physical fitness.

6.2.2. Methodology

The Freeman Hospital, Newcastle upon Tyne, is the North-East of England's transplant centre, and one of seven within the UK. Throughout the year, transplant assessment meetings (TAMs) are held fortnightly to assess patients presented for referral to the active transplant waiting

list. These meetings are held involving a multidisciplinary team including hepatologists, transplant coordinators, dieticians, social workers and anaesthetists where information obtained from each clinician/health care professional (HCP) is combined to provide a fully informative dataset for each patient to allow a decision on listing to take place.

6.2.3. Data collection

Data were collected from all patients being assessed for liver transplantation at The Liver Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, UK between January 2014 and March 2018. Each patient was discussed at a multidisciplinary TAM to determine their suitability for liver transplantation. Data from these meetings were used as the primary data source for this cohort study (n=332). The study protocol was approved by North East-Newcastle & North Tyneside 1 Research Ethics Committee (REC reference: 16/NE/0144). The data obtained related to all patients assessed for suitable for wait-listing for liver transplantation, however, this was largely to inform the development of the exercise programme targeted towards people who were considered suitable candidates to receive a liver transplantation and subsequently referred to the active waiting list. While the data relating to that of the patients who were not wait-listed was of interest, the primary goal was to describe the clinical characteristics of those who would receive the intervention, i.e. those on the active waiting list.

6.2.4. Data analysis

Data collected for each patient included demographics and underlying aetiology of liver disease (Table 6.1); lifestyle behaviours (alcohol, smoking, physical activity (PA)), weight, BMI and measures of functional fitness (Anaerobic Threshold (AT), peak oxygen consumption (VO_{2peak}) and grip strength) (Table 6.1). The underlying aetiology of a patient's liver disease was defined as the initial diagnosis of disease affecting the liver. Patients with hepatocellular carcinoma (HCC) were recorded and grouped separately to other aetiologies in addition to being grouped into their underlying liver disease. Blood results were taken from the patients' most recent blood profile.

6.2.5. Lifestyle and functional measurements

Information relating to lifestyle behaviours (alcohol consumption and smoking status) was collected through patient self-report and psychosocial evaluation by the medical and

addictions team. A cardiopulmonary exercise test (CPET), performed on a cycle ergometer using a Wassermans protocol, was used to obtain data on AT and VO_{2peak} (Wasserman et al., 2005). Not all patients were able to reach AT or VO_{2peak} for reasons other than poor fitness, and these reasons were recorded (e.g., large volume ascites). Grip strength (Takei 5401 digital dynamometer) was measured as an indicator of frailty (Xue et al., 2011), on both hands. The score from the patients' dominant hand was recorded.

While some patients were formally asked about their levels of physical activity (PA), responses ranged from self-assessed levels of independence to maximum distance they were able to walk/push themselves in a wheelchair. Therefore, a homogeneous PA data set was not available for analysis.

6.2.6. Statistical analysis

Descriptive statistics were used to define the whole cohort who were assessed for suitability for liver transplantation. Normality was assessed using box plots and the Shapiro-Wilk calculation. Descriptive statistics were used to compare patients' subcategorised into listed/not listed for transplant. Subsequently, the descriptive statistics for the 'listed for transplant' and 'not listed for transplant' sub-groups were compared. The unadjusted analyses of differences for categorical variables (listed and non-listed) were performed using the Pearson's Chi-squared test and the Independent t-test for continuous variables, with p values <0.05 considered statistically significant. All statistical analyses and comparisons were undertaken using SPSS Version 24.0 (IBM Corp, 2018).

6.3. Results

6.3.1. Patient characteristics

Patient characteristics are presented in Table 6.1. The overall cohort that was assessed had a median age, weight and BMI of 58 years, 79 kg and 27 kg/m^2 respectively. Similarly, within the overall cohort, 63% of all patients assessed were male, and there were no significant differences in the male/female ratio between the listed and non-listed sub-categories. There were no significant differences in age, gender, weight or BMI between the listed and non-listed sub-categories.

6.3.2. Liver disease characteristics

Across the whole patient cohort, Alcoholic Liver Disease (ALD) was the most common aetiology requiring assessment for transplant (Table 6.1). Autoimmune (AI) liver disease (including autoimmune hepatitis, primary biliary cholangitis and primary sclerosing cholangitis) and non-alcoholic fatty liver disease (NAFLD) showed statistical significance between the listed and non-listed groups (p=0.004 and p=0.04 respectively), with more patients being listed than not in the AI group, and less patients being listed within the NAFLD group. Other aetiologies showed no variance between groups.

The most common associated comorbidity was type 2 Diabetes Mellitus (T2DM) with an overall prevalence of 30%, ranging from 15% amongst those with AI liver disease to 75% of those with NAFLD. Cardiovascular disease (CVD) affected 18% of patients, and chronic obstructive pulmonary disease (COPD) affected 8% of the total cohort. When compared to the non-listed group, listed patients had a significantly higher Model for End Stage Liver Disease (MELD) (p=0.001) and United Kingdom Model for End Stage Liver Disease (UKELD) score (p=<0.001), Bilirubin (p=0.003), Alanine Transferase (p=0.008) and Alkaline Phosphate (p=0.001) (Table 6.1). See appendix L for normative MELD and UKELD values.

6.3.3. Lifestyle characteristics and functional capacity

Overall, 17% of patients assessed for suitability for liver transplantation reported smoking regularly. There was a lower prevalence of current smokers in the listed compared to the non-

listed group (p=0.001) (Table 6.1). In the context of alcohol, when patients with ALD were excluded from the analyses (i.e. patients with ALD are required to be abstinent from alcohol before consideration for transplantation), there was no significant difference in alcohol consumption between the listed and non-listed groups, with 18% of all patients assessed reporting consuming alcohol.

Mean AT amongst all patients assessed for suitability for liver transplantation was 12 ml/min/kg. There was no significant difference in AT between the listed and non-listed groups. This could be largely attribuTable to a significantly higher proportion of patients who were unable to start the CPET in the non-listed group, therefore not generating a measurable value for AT (Table 6.1). In situations where patients were unable to start the CPET, this was due to a lack of fitness in 24% of cases, and in these cases patients reported difficulty getting on to the bike or struggled to pedal at minimal resistance. 15% of patients were unable to start the CPET due to their comorbidities (e.g. musculoskeletal conditions). Other reasons for patients not being able to start the CPET were ascites or being "too unwell". For 44% of patients unable to start their CPET, there was no reason recorded. There was no difference in grip strength between groups, with overall grip strength from all patients being reported as 25 kg.

6.3.4. Non-listed population (See Figures 6.1 and 6.2)

The most common reason reported for patients not being listed was deferral, often necessitating an opinion from another clinician or healthcare professional, or requiring further investigation (e.g. coronary angiographies, endoscopies or further imaging). This was classified as 'requiring further investigation' (30%) (Figure 6.1).

The requirement for lifestyle modification was the next most common reason for not listing (23% of patients) (Figure 6.1). Of the 39 patients within this category, 56% were advised to improve their fitness and/or to reduce their BMI; 15% of patients were advised to stop smoking; and 15% were advised to abstain from alcohol (Figure 6.2). Remaining patients required nutritional reconditioning or increased psychological support (13%).

Table 6.1. Characteristics of underlying liver disease, associated complications, comorbidities, lifestyle behaviours and functional capacity.

Categorical data are presented as %. Continuous variables are presented as median (range).

	Total (n=332)	**Listed (n=157)**	**Non-listed (n=175)**	**P**
Age	58 (17-74)	58 (17-72)	58 (24-74)	0.562
Weight (kg)	79 (44-146)	82 (48-146)	77 (44-137)	0.069
BMI (kg/m^2)	27 (18-47)	26 (18-46)	28 (16-47)	0.724
Gender: Male (%)	63	68	59	0.079
Aetiology:				
ALD (%)	39	36	43	0.217
NAFLD (%)	15	11	19	0.040*
NAFLD + ALD (%)	5	5	5	0.824
Autoimmune liver disease (%)	18	25	13	0.004**
Cryptogenic cirrhosis (%)	4	3	4	0.691
Hepatitis (%)	6	6	5	0.813
Other (%)	14	15	13	0.581
Hepatocellular Carcinoma (%)	17	15	20	0.355
Comorbidities				
T2DM (%)	30	28	31	0.572
CVD (%)	18	15	21	0.212
COPD (%)	8	6	9	0.348
Blood parameters				

Bilirubin (umol/L)	37 (11-287)	48 (13-287)	31 (11-41)	0.003**
Albumin (g/L)	34 (28-45)	34 (31-38)	34 (28-45)	0.595
Creatinine (umol/L)	76 (65-176)	74 (65-176)	78 (71-116)	0.780
Alanine aminotransferase (unit/L)	31 (16-79)	35 (19-79)	26 (16-47)	0.008**
Urea (mmol/L)	5.2 (3.9-16.5)	4.8 (3.9-16.5)	5.5 (4.4-8.8)	0.140
Alk Phosphate (unit/L)	147 (73-869)	152 (73-869)	141 (116-266)	0.001**
Hb (g/L)	110 (81-149)	112 (81-129)	109 (125-149)	0.523
Severity of Liver Disease				
MELD	14 (1-40)	16 (6-40)	13 (1-40)	0.001*
UKELD	53 (38-75)	55 (38-71)	53 (42-75)	<0.001*
Alcohol (Excluding ALD)				
Consumes alcohol (%)	18	23	14	0.093
Does not consume alcohol (%)	37	31	42	0.176
Abstinent for >6 months (%)	25	27	23	0.505
Abstinent for <6 months (%)	7	6	7	0.774
Unknown (%)	14	13	15	0.121
Smoking status				
Smoker (%)	17	10	24	0.001*
Non-smoker (%)	54	59	49	0.085
Quit > 6 months ago (%)	24	28	21	0.145
Quit < 6 months ago (%)	2	2	2	0.812
Unknown (%)	2	1	3	0.201
Functional Capacity		Listed (n=118)	Non-listed (n=112)	
Anaerobic Threshold (n=230)	12±3	12±2	11±3	0.454
$VO_{2peak)}$ (ml/kg/min) (n=223)	14.4±5	15±5	14±4	0.897
Didn't start CPET (%)	14	10	18	0.032*

Did not complete CPET (%)	11	11	10	0.872
Completed CPET (%)	77	80	73	0.127
Grip strength (kg) (n=71)	25±9	28±11	24±8	0.466

**Denotes statistical significance at p<0.05; ** Denotes statistical significance at p<0.01*

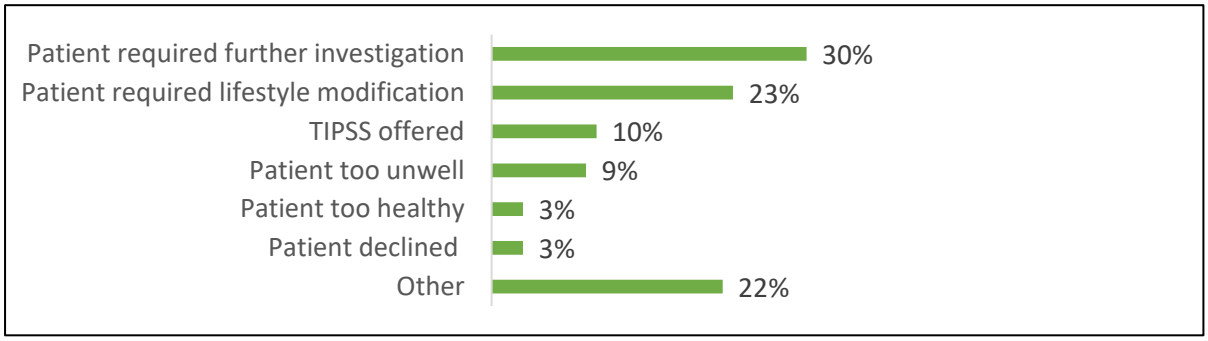


Figure 6.1. Reasons for ineligibility for liver transplantation

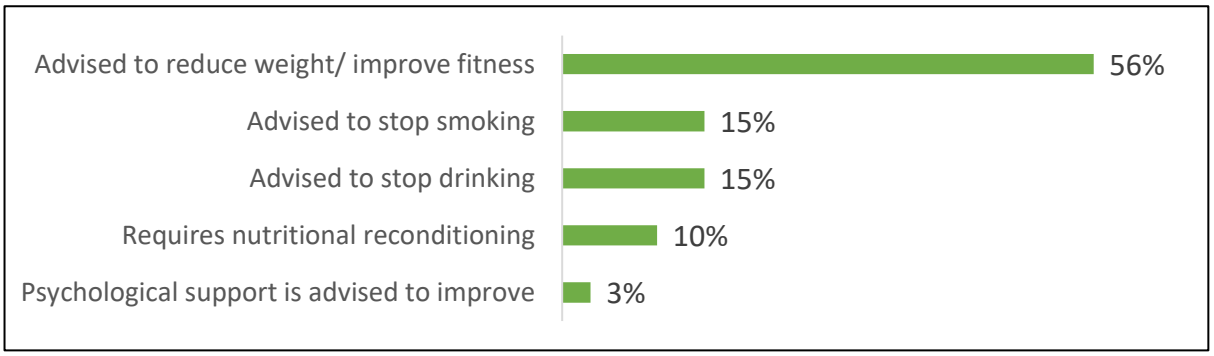


Figure 6.2. Required Lifestyle Modifications in order to be eligible for liver transplantation

6.3.4.1. Listed Population

Of all patients listed for transplant, the median age, weight and BMI respectively were 59 years, 81 kg and 27 kg/m^2 representing an overweight cohort of patients. 68% of the listed cohort were male. When grouping the underlying aetiologies of patients, the cohort analysis revealed the most common aetiology in those who are listed was ALD at 36%, with the second and third most prevalent being autoimmune and NAFLD, with 25% and 11%, respectively. 16% of listed patients had HCC and 39%, 38% and 25% had developed varices, ascites and encephalopathy, respectively. There was a high prevalence of comorbidities, with 32% of listed patients diagnosed with T2DM and 30% had a diagnosed MSK condition. The median MELD and UKELD scores were 15 and 34, respectively. In terms of patients listed for transplantation, 23% reported regularly drinking alcohol (excluding patients with ALD as an underlying aetiology) and 9.9% reported still smoking. Cardiorespiratory fitness was low, with median scores of 12 ml/kg/min (AT) and 14 ml/kg/min ($VO_{2peak)}$) reported. The completion rate for CPET was 81%, with the remaining patients either being unable to initiate the CPET (8%) or being unable to complete it (11%). Median grip strength was recorded at 26 kg, which represents a normative value for a female over the age of 60 years old (Massy-Westropp et al., 2011). Therefore, it can be interpreted that the patients assessed as part of this cohort analysis were generally below the normative grip strength value for both their age and gender.

6.3.4.2. Non-listed Population

Patients who were ineligible to be listed for liver transplantation had a median age, weight and BMI of 58 years, 75 kg and 27 kg/m^2, respectively, and 59% of this population were male. As with the listed population, the most common aetiology was ALD (39%), with AIH (18%) being the second most common and NAFLD (15%) being the third. 20% of non-listed patients had HCC, and 47% had ascites, 46% had varices and 22% had encephalopathy. Within this population, the most common comorbidity was MSK (32% patients), and T2DM was the second most common comorbidity (31% of patients). The median score for AT was 11, and 14 ml/kg/min for VO_{2peak}. Completion of the CPET was 72%, with the remainder of patients being unable to initiate CPET (18%) or being unable to complete it (10%). Median grip strength

was recorded as 24 kg, which, as previously discussed, falls considerably lower than normative grip strength value for patients of this age and/or gender (Massy-Westropp et al., 2011).

When patients with ALD were excluded, there was no significant difference in alcohol consumption between listed and non-listed populations (p=0.093). There was a lower prevalence of smokers in the listed group compared to the non-listed group (p=0.009).

There was no significant difference between AT or peak VO_2 between listed and non-listed patients (p=0.454). This could potentially be explained by the large amount of missing values for the non-listed group due to inability to participate in the CPET, which was significantly higher in the non-listed group (p=0.009). The inability to participate in the CPET was often due to a lack of fitness, a complication associated with liver disease or a comorbidity related problem in both listed and non-listed populations. Similarly, grip strength showed no statistical difference between cohorts (p=0.455). However, the median recorded grip strength of 25.75 kg is considered very poor for both males and females aged 58 (average age) (Massy-Westropp et al., 2011). On a normative scale, a peak VO_2 of 14.45 ml/kg/min would be regarded as 'very poor' in both males and females (Rangari et al., 2019).

Approximately 15% of patients were unable to start the CPET due to their comorbidities. In the vast majority of patients, this was MSK related, usually due to back, hip or knee pain. This was similar for those who were able to start but unable to complete the CPET, where often they would report pain part way through the exercise necessitating them to stop before reaching their AT. This was consistent across listed and non-listed patients, and therefore showed the exercise capability of potential intervention recipients.

6.3.5. Discussion

The aim of this cohort analysis was to define the characteristics and functional capacity of patients being assessed for liver transplantation in order to ultimately inform the development of an exercise intervention for patients on the active waiting list. However, depiction of the non-listed group presents an interesting group of patients, who likely no longer have any viable treatment methods remaining for their liver disease, as liver transplantation is the only curative therapy for those with decompensated cirrhosis (Cholongitas et al., 2006). Therefore, it is vitally important that as many patients as possible

are put forward for liver transplantation, are eligible. In this respect, it is imperative to characterise this sub-group of the cohort in an effort to understand why they are not considered a suitable recipient for liver transplantation and to maximise the functional capacity of those awaiting transplant.

Given the significant ongoing disparity between the supply of liver grafts and the demand elicited by individuals on liver transplantation waiting lists (NHS, 2019, Network, 2017), it is important to ensure that those who are offered the surgery are in the best health possible. This is of considerable importance for patients who are both waitlisted and not waitlisted. Specifically, patients who are offered the transplant should aspire to be in the best possible health to maximise the longevity of the transplanted liver, and minimise post-operative adverse events (Prentis et al., 2012, Levesque et al., 2017, Yadav et al., 2015). It is well reported that patients experience a significant decline in functional capacity while on the waiting list (McAdams-DeMarco et al., 2019, Duarte-Rojo et al., 2018), which has a subsequently negative impact on post-operative mortality and functional capacity (Prentis et al., 2012, Kaido et al., 2017). Indeed, regardless of age, frailty has recently been associated with almost a two-fold increased risk of waitlist mortality (Haugen et al., 2020). Furthermore, given that there is a significant risk of developing metabolic syndrome following liver transplantation (Dunn et al., 2020), it is important to implement active lifestyle behaviours pre-transplantation in order to better facilitate the patients return to exercise/ activity post-transplant. Indeed, considering only 40% of patients achieve physical robustness 1 year after liver transplantation (Lai et al., 2018) and only 25% are physically active (Painter et al., 2001), it is essential to encourage as many pre-transplant patients as possible to remain active in order to better prepare them for a return to everyday activity. A structured physical activity/ exercise regime devised prior to transplantation would likely provide patients with a framework for resuming activity.

For patients who are not waitlisted, it is still important to maintain as good a level of physical activity/ functional capacity as possible in order to reduce liver disease associated complications and maintain a good quality of life (Tapper et al., 2015). Furthermore, from a clinical perspective, understanding reasons for ineligibility for waitlisting is of paramount importance going forward, to ensure that patients with the most optimum projected outcomes are offered transplantation. Therefore, it can be assumed that maintenance of

physical activity will serve to increase longevity in the absence of liver transplantation in this sub group of patients.

6.3.5.1. Non-listed population

The main findings of the retrospective cohort analysis were, all patients assessed for suitability for liver transplantation, regardless of whether they were waitlisted or not, had extremely poor fitness and poor musculoskeletal strength (indicated by grip strength). With regards to the non-listed population, 23% of patients assessed for liver transplant required lifestyle modification before being listed; and 56% of these non-listed patients were advised to improve their physical fitness and reduce weight. There was a significantly higher proportion of patients who could not start the CPET in the non-listed group. Following an unsuccessful transplant assessment, non-listed patients face a poor prognosis (Kress et al., 2000). A high proportion of these patients could potentially be listed if tailored interventions were provided to target lifestyle behaviour change prior to assessment for liver transplantation.

One of the primary reasons for non-listing within the patient cohort was the requirement for lifestyle modification prior to listing. These data highlight a growing need for optimisation of lifestyle behaviours *prior* to assessment for transplant, and suggests that lifestyle interventions should be integrated within clinical care pathways to increase the number of patients eligible for listing and to optimise outcomes of transplant long-term. There is no literature comparing the fitness of listed and non-listed patients, however, previous research has reported on measures of functional capacity in patients undertaking assessment for suitability for liver transplant. For example, VO_{2peak} in cirrhotic patients with compensated clinical disease status has been reported as 20 ml/kg/min (Campillo et al., 1990) which varies significantly from the findings of this cohort analysis (15 ml/kg/min and 14 ml/kg/min for listed and non-listed respectively). A study in 2015 reported a VO_{2peak} of 22 ml/kg/min in patients awaiting liver transplant within a cohort of 8 patients (Debette-Gratien et al., 2015). Lai et al (2013) have shown that patients who are unable to complete CPET or obtain an AT score have an increased likelihood of a poor outcome following major surgeries, specifically longer hospital stays and higher early and medium-term mortality (Lai et al., 2013). The data from the current cohort analysis shows that 18% of all non-listed patients were unable to

complete the CPET, compared with 10% of those listed. The proportion of patients unable to complete the CPET due to inadequate fitness reflects a sub-group of patients too unfit to undergo liver transplantation, or at a greater risk of poor post-transplant outcomes. In the cohort, this represents 10% of all patients assessed. This presents a unique group of patients who would benefit from lifestyle optimisation prior to transplant assessment, to increase the efficacy of the assessment procedure. Identifying those unlikely to complete the CPET early in the assessment procedure would allow timely provision of a physical activity/exercise intervention, tailored to individual needs and capabilities to improve fitness.

Non-listed patients face a poor prognosis following unsuccessful assessment for liver transplantation and a higher likelihood of mortality. Previous research has reported a significant difference in mortality of patients with advanced liver disease who were refused transplantation when compared to those who were successfully transplanted, with an overall mortality of 56% compared to 12%, respectively (Kress et al., 2000). There is limited follow-up data reporting on the prognosis of those refused transplant, but given the significant difference between successful and unsuccessful liver transplant recipients, and the projected increased demand for liver transplantation (Arulraj and Neuberger, 2011) it is becoming increasingly important to target lifestyle optimisation prior to assessment for listing.

The non-listed population described in this cohort analysis would benefit significantly from prehabilitation prior to being assessed for suitability for transplantation. Should these patients engage in prehabilitative exercises in order to maximise pre-assessment fitness (or lose weight), this could represent a significantly larger proportion of patients who are eligible for transplantation. Subsequently, this would act to streamline the assessment procedure, thereby making it a more time and cost effective process.

6.3.5.2. The role of the cohort analysis for informing the exercise intervention

The cohort analysis indicated that the target population of the intervention would comprise of very unfit patients, with measures of functional fitness comparing poorly to healthy age/gender matched individuals within the general population. This demonstrated a need for an exercise intervention to target cardiorespiratory fitness and strength, to improve post-transplant survival. Currently, there are established pathways in place to target smoking and alcohol cessation, however there are no agreed, evidence-based dietary or PA/exercise

interventions integrated within the clinical pathway that target pre-transplant weight loss and fitness in listed patients.

Evidence is emerging that exercise training in solid-organ transplant recipients is a safe and effective approach for improving health pre-transplant (Wallen et al., 2016). Importantly, supervised exercise training in liver transplant candidates has shown to be safe and feasible, particularly in obese cirrhotic patients (Williams et al., 2019, Macías-Rodríguez et al., 2019, Spengler et al., 2017) improving hepatic venous pressure gradient and ventilator efficiency, with no episodes of hepatic encephalopathy or variceal bleeding reported (Macías-Rodríguez et al., 2016). The increased reassurance provided from these studies has served to pave the way for implementation of structured prehabilitative interventions in patients on the liver transplant waitlist.

Prehabilitation in advance of cardiovascular, lung and digestive surgeries have been reported to improve pre-operative VO_{2peak}, with a recent meta-analysis reporting how prehabilitation could reduce postoperative complications following abdominal surgery (Moran et al., 2016). Interventions comprising supervised cycling ergometery, kinesiotherapy and treadmill walking (Zenith et al., 2014, Román et al., 2016, Macías-Rodríguez et al., 2016, Román et al., 2014) have assessed the effect of prehabilitative exercise interventions on 1 year survival and complication rate post-transplant, as well as VO_{2peak}, liver function and body composition. All four studies concluded that supervised exercise was safe and efficacious in the context of increasing exercise capacity (Román et al., 2014, Zenith et al., 2014, Román et al., 2016), VO_{2peak} (Macías-Rodríguez et al., 2016, Zenith et al., 2014), quality of life (Román et al., 2014) and muscle mass (Román et al., 2014, Román et al., 2016, Zenith et al., 2014). Furthermore, no studies have reported cirrhosis decompensation or adverse outcomes during the exercise interventions.

The cohort analysis presented a range of patients with varying comorbidities. Specifically, those of a MSK nature were prevalent, therefore identifying the need for a versatile intervention, capable of accommodating patients' needs. This is important because it was reported to be a significant barrier to starting and completing the CPET and therefore would likely limit patient participation in an exercise intervention. However, the wide range of comorbidities reported presents a cohort of patients that need a flexible intervention that could be adapted to facilitate participation despite patients being restricted due to their

comorbidities. Similarly, it was noted that consideration should be given to liver-related complications, such as ascites, varices and encephalopathy. This informed the development of the exercise intervention, because it became apparent that patients with significant ascites or varices would require modifications to their exercises to take part safely (Tandon et al., 2018). Modifications could include; ensuring adequate primary or secondary variceal prophylaxis is in place prior to exercise participation (for patients with significant varices) and having caregiver supervision for those with significant ascites and subsequent impaired balance (Tandon et al., 2018). An alternative approach recommended for managing exercising with ascites is to progress exercises on days where ascites accumulation is insignificant and/or does not affect balance. With regards to patients with significant hepatic encephalopathy, it is recommended that medical optimisation is in place to manage the symptoms of encephalopathy prior to partaking in any exercise, and that exercises are supervised by caregivers, or if not possible, a certified exercise professional (Tandon et al., 2018). Other modifications that could be utilised include the use of supported, or chair-based exercises, for patients who are at a greater fall risk, to minimise the risk of injury during exercise sessions.

The cohort analysis highlighted the importance of an intervention that is adapTable and flexible that could be easily personalised according to each patient's physiological needs, given the range of co-morbidities within this cohort. As such, it was imperative that the intervention should involve a variety of exercises, targeting cardiorespiratory, muscular strength and stability. Furthermore, this data supported previous research that has described the large proportion of patients with cirrhosis who have sarcopenia, i.e. estimates ranging from 22-62% (Meza-Junco et al., 2013, Montano–Loza et al., 2012, Masuda et al., 2014, Van Vugt et al., 2016). Furthermore, sarcopenia is independently associated with both waiting list and post-liver transplant morbidity (Van Vugt et al., 2016, DiMartini et al., 2013, Englesbe et al., 2010). This further describes the need for exercise within this cohort of patients, but also presents a further consideration that should be employed when developing an exercise intervention for patients with cirrhosis.

Significant modifications and considerations, are required when 'prescribing' exercise to the liver transplant population due to the variance of the underlying aetiologies and prevalence of comorbidities. This represents the need for an intervention that could be delivered in a

variety of settings and environments, and not dependent on the availability of any particular exercise equipment. For example, in order to undertake supervised exercise, patients may exercise in a range of locations- at home, at a friend or family members home or within a community exercise setting. This cohort analysis was therefore essential for providing important information about frailty and fitness of the cohort to whom the exercise intervention was targeted (i.e. a significant proportion had low fitness levels while awaiting liver transplantation). This information highlighted that an intervention was imperative to improve the fitness of all patients on the liver transplantation waitlist.

6.3.6. Conclusion

This cohort analysis described the characteristics and functional capacity of patients who were assessed for inclusion onto the liver transplant waiting list. It described a cohort with relatively poor functional capacity, and given the important role that perioperative fitness has on post-transplant outcomes, affirmed that an exercise intervention would be beneficial for patients awaiting surgery. It provided insight into the breadth of comorbidities that was essential to understanding the target patient population, while at the same time highlighting the considerations required when designing an exercise intervention.

6.4. Phase two: development of the exercise intervention

6.4.1. Introduction

AASLD guidelines (Burra et al., 2016, Murray and Carithers Jr, 2005) and an extensive variety of a research (Prentis et al., 2012, Lai et al., 2013, Lai et al., 2014, De Luca et al., 2015) have described the imperative need for a structured exercise programme for patients awaiting liver transplantation. However, this presents the need to develop an intervention that is acceptable to both patients and healthcare professionals (HCPs) involved in delivering their care, particularly in terms of the mode of delivery.

There are currently limited exercise programmes available for patients awaiting liver transplantation. A plethora of research studies have focussed primarily on exercise programmes for patients with chronic liver disease (Hallsworth et al., 2011, Debette-Gratien et al., 2015, Román et al., 2016, Williams et al., 2015, Zenith et al., 2014, Berzigotti et al., 2017, Kruger et al., 2018, Nishida et al., 2017), but not specifically for patients awaiting transplantation. Recently, it has been reported that the use of exercise training in patients with cirrhosis has improved cardiopulmonary fitness, metabolic syndrome, hepatic venous pressure gradient, sarcopenia and health related quality of life (Duarte-Rojo et al., 2018). Furthermore, when surveyed, 87% of hepatologists recognised the importance of exercise and reported routinely assessing whether patients exercised regularly, educating them about its benefits and providing specific exercise recommendations (Duarte-Rojo et al., 2018). The safety of exercise in patients waiting solid organ transplantation has been recently evaluated, and while modifications should be employed, overall tailored exercise has been shown to be safe in these patient populations (Wallen et al., 2016). Despite the recognised safety and benefits of exercise prior to transplantation, there are no standardised recommendations on exercise intervention whilst on the liver transplant waiting list. Barriers to acceptability of community based programmes delivered face-to-face for patients awaiting liver transplantation include; ill health, impractical travel times and associated costs, and family/work obligations (Webb et al., 2019, Jones et al., 2007). This is unsurprising, as many patients travel significant distances to their regional liver transplant centre.

The feasibility of a home based exercise programme has recently been assessed in patients awaiting liver transplantation (Williams et al., 2018, Williams et al., 2019) and was reported to be acceptable to participants (n=20) and feasible to deliver. The intervention involved resistance exercises and incremental daily step count targets that resulted in improvements in AT and measures of functional capacity. However, the authors did not incorporate patient views in the development of the intervention.

When evaluating potential interventions aimed at patients with significant clinical burden, previous studies have indicated the importance of forming a collaborative relationship with the target population (Connell et al., 2015). This is essential as it facilitates and enables structured discussions to understand current problems, motivations for uptake/participation and barriers and facilitators to continued engagement. This process increases the likelihood that behaviours (in this case exercise) can be appropriately targeted by a theory and evidence-informed intervention that can be robustly evaluated.

The overall aim of phase 2 was to develop an exercise intervention for patients who have been wait-listed for liver transplantation. This involved a series of focus group discussions of which findings would be combined with those from the retrospective cohort analysis to inform key intervention decisions. It was hypothesised that patients in attendance at the focus groups would find the idea of an exercise programme acceptable and would inform the development of such a programme.

6.4.2. Methods

A four stage intervention development process (Figure 6.1) was undertaken to co-design the exercise intervention and to discuss acceptability and usability of the intervention for people awaiting liver transplant. The developmental stages were as follows:

- Stage 1- Exploratory work (including the cohort analysis presented in section 6.2) to characterise the lifestyle behaviours of the target population (patients assessed for liver transplantation).
- Stage 2- Interactive focus group discussions with patients and HCPs to inform the content of the exercise intervention
- Stage 3- Development of a prototype exercise intervention

Figure 6.3 highlights the pathway of the development of the intervention.

The aim was to develop an exercise intervention that was suitable for patients on the waiting list for liver transplantation. It was important that the intervention could be delivered through existing clinical pathways, potentially through a referral system during clinical appointments. It was also important that the developed intervention would allow appropriate progressions and regressions for patients, thereby meaning the exercises can be adapted to the needs and capabilities of patients. The study protocol was approved by North East-Newcastle & North Tyneside 1 Research Ethics Committee (REC reference: 16/NE/0144).

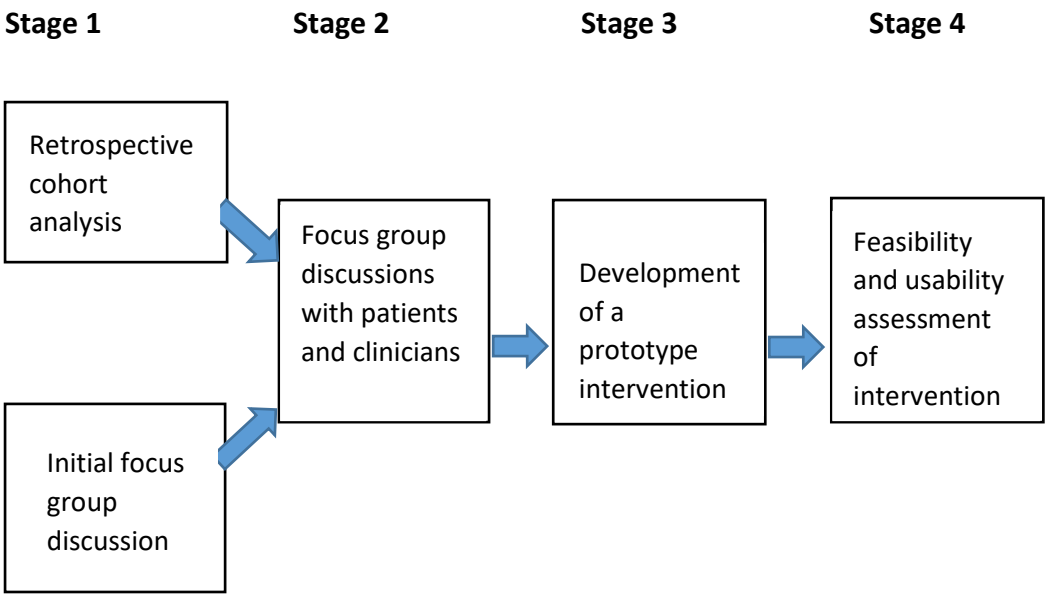


Figure 6.3. Summary of the intervention development process

6.4.2.1. Stage 1: exploratory work

An observational study conducted in 2012 reported that pre-transplantation fitness is associated with perioperative 90-day survival (Prentis et al., 2012). Similarly, more recent research has indicated that poor cardiorespiratory reserve is associated with increased 1-year mortality (Bernal et al., 2013). Our cohort analysis demonstrated that fitness levels of patients on the active waiting list, while varied, were still generally poor compared to the standard population. As such, it was highlighted that an exercise intervention was required to improve fitness levels of patients pre-transplantation. Collectively, these studies emphasise the importance of exercise and outcomes post liver transplantation. However, liver transplantation is not associated with improvements in levels of exercise (Dharancy et al., 2008). Currently there are no structured care pathways/interventions to target exercise in this patient population. The cohort analysis described previously enabled a description of the liver transplant waitlist population, including fitness levels (See section 6.2).

To complement the findings of the cohort analysis, a preliminary focus group discussion was conducted with patients at Freeman Hospital, Newcastle upon Tyne, UK to determine whether participation in an exercise intervention was something they would be interested in and potentially engage with. Focus group participants were purposefully selected by a clinician and included patients and healthcare practitioners. Specifically, patients were selected to represent different stages of the assessment and wait-listing procedure (for example, currently on the waiting list and having experienced time on the waiting list and since received a liver transplant), to ensure males and females were represented and to represent different underlying aetiologies of liver disease. Purposefully selected staff members were identified and invited to partake with the aim of representing a range of staff involved in the waitlisting pathway. For example, anaesthetists and/or hepatologists who might play an executive role of making decisions with regards to the patients' clinical care and transplant coordinators to represent the patients' primary point of contact with their transplant healthcare team. Furthermore, a patient representative from LiverNorth was invited to attend the focus group in order to have someone with experience of working with HCPs and who was comfortable engaging with medical/research staff to help ensure that all patients' views were heard. The patient representative was also invited to attend due to their

lived experience of co-producing interventions with patient and public involvement and therefore could reflect on what had worked well in the past and how best to ensure an intervention was developed in line with patient needs.

6.4.2.1.1. Results: focus group discussion

A purposive sample of four patients (one wait-listed and three transplant recipients) and four clinicians, (two health psychologists, a consultant anaesthetist, a transplant coordinator, and a representative of LiverNorth) were invited to attend a focus group. Characteristics of attendees are described below. The primary aim of the focus group discussion was to obtain important information to inform exercise intervention content, design and delivery.

A topic guide (appendix M) was used to guide discussion. Prompts were used to probe for further information where required, including views on whether a home-based, community, or hospital/clinic based exercise intervention would be more desirable and to discuss barriers and enabling factors to participation and completion of an exercise intervention at each of these settings. The focus group discussion was audio recorded, transcribed verbatim and analysed using thematic analysis.

6.4.2.1.2. Characteristics of attendees of the initial focus group

A purposive sample of patients were invited to attend the focus group discussion based on transplant status (e.g., waitlisted, transplanted) and underlying liver disease aetiology. This was the primary criteria for purposive sampling, so that representation of a range of patients who may receive this intervention was achieved. Similarly, sampling attendees based on transplant status meant that current patients' needs could be considered, as well the views of patients who could reflect on the entire time that they had spent on the transplant waiting list. Four patients attended, three of which were male, within an age range of 50-62 years. One patient was on the active waiting list at the time of the focus group, and the other three had previously received a liver transplant. Of those who had received a liver transplant, two were in receipt less than 10 years ago, and the other >20 years ago.

Members of staff in attendance at the focus group comprised of a transplant coordinator, two health psychologists, a consultant anaesthetist and a patient representative from Liver North.

Following thematic analyses of the data obtained from the initial focus group, seven themes were generated, as summarised below in Table 6.2.

Table 6.2. Themes and sub-themes obtained from thematic analysis of focus group discussion.

Theme/ sub-theme	**Supporting quotes**
Patients	
Information about the importance of exercise in relation to my liver transplant would motivate me to engage in an exercise intervention	*"Information would actually probably encourage me, I'm still thinking about buying a bike to do some exercise because I cannot run"*
I feel/felt capable of undertaking exercise while on the active waiting list	*"I did about 7,000 [steps] this morning"* *"I walk on the beach, like I say"* *"See at the minute, the thing I do, I work still and it's a very physical job I do so I'm very active all the time really"*
Safety is important to me when undertaking exercise	*"[I could] try it at home and say, "I think I'd like someone watching over me,"… I'm frightened of falling."* *"I think it feels safer if you're with someone watching you."*
Social support is a significant motivator to maintain adherence to an exercise intervention	*"When people do things in groups, I think it gives them more support and I think they do more"* *"If you could do it together, it might have be better"* *"It's a social thing as well."*
HCPs	

It is important to have an exercise intervention that can be individualised	*"We have a lot of patients with a lot of variance so we have some people that are really, really quite fit and quite well and then we have, equally, some people that are really struggling a little bit and who struggle to do normal things like make a cup of tea"*
Patients should be able to do the exercises anywhere	*"It needs to be local to where you are"* *"[It could be] home, hospital or community"* *"So you could maybe take what's given to you to a leisure centre and do it there"* *"So could we do you get the opportunity to say you can do this at home if you wanted or you could go to a gym to do it supervised and we could get you in to do that. Then that gives the individual choice about whether they want to do it at home, whether they want to do it in the community"*
Difficulty accessing a central location to attend training session could be a barrier to uptake	*"It's only once every six months I come here, the appointments are split between Middlesbrough and here… It's a bit far to come"*

Patients

Information about the importance of exercise in relation to my liver transplant would motivate me to engage in an exercise intervention

Patients reported being motivated to participate in an exercise intervention:

"I think it's a great idea but I think you've got to look at resources and be realistic"

Information or education about the importance of preoperative fitness was reported to be a motivating factor to participation (i.e. the role that it played in the liver transplant process):

> *"In my mind after [being put on the waiting list] was, "I've got to stay like this. I can't afford to drop back and get sick in some other way where I would not be any longer on the list or ready for a transplant."*

Prior to the focus groups, not all patients were aware of the importance of preoperative fitness and this information provided an incentive for these individuals to increase their fitness. Patients who were previously liver transplant recipients reported feeling that exercise while on the waiting list would have been well received once they knew the benefits.

I feel/felt capable of undertaking exercise while on the active waiting list

Liver transplant recipients reported feeling capable of undertaking light exercise for the vast majority of the time spent on the waiting list. This was the consensus among recipients and those listed at the time of the focus group:

> *"It would have been brilliant…to have this when I was waiting for [my transplant]"*

> *"Before my transplant, I did no activities whatsoever [then] the transplant coordinator came and said, 'Can you do volley ball?'…and by doing that, I started playing volley ball, doing 5k and 10k walks"*

Safety is important when undertaking exercise

All patients in the group reported being aware of their own limitations with regards to exercise, but were motivated to improve, or at least maintain their current fitness levels:

> *"[I] want to maintain that level of fitness"*

However, patients were also cautious about exercising alone or at home mainly due to concern about not being able to motivate themselves or performing the exercises correctly and/or safely:

"You're supervised, you're shown how to do that [exercise]… Show that you're safe, show that you can do it and then over to the home based element"

Social support is a significant motivator to maintain adherence to an exercise intervention

Safety issues were of concern to the majority of patients, therefore the consensus was that an intervention may be more acceptable in a community-based setting, with the support of others in the same or similar situation. This was reported to be more motivating. Patients also reported that the time spent on the waitlist was a difficult time, with multiple patients reporting feelings of anxiety and guilt, pre- and post-transplant. Despite that, an exercise intervention would still be well received due to the potential benefits such as improved preoperative fitness, functional fitness and stability, and patients reported being motivated to have community-based exercises to ensure ongoing participation:

"If you've got somebody encouraging you... You always need somebody to just give you that little extra push"

Health Care Professionals

It is important to have an exercise intervention that can be individualised

Participating healthcare professionals provided valuable insights regarding the mode of delivery and content of an exercise intervention for liver transplant patients. In terms of content, they reported the wide range of variance in the disease state and therefore emphasised the importance of having varied content that can be widely applied to a range of patients. Specifically, it was reported that the patients with encephalopathy may not be stable on their feet and therefore recommended floor/chair-based exercises for those patients, particularly if they wanted to exercise at home:

"People whose minds are a bit foggy just because of their liver disease… maybe they need to do more floor based exercise so we're not going to have them falling over"

This helped to inform the safety of the intervention, i.e., further safety measures were recommended (e.g., safety mats and having someone with patients at all times while completing exercise and the availability of chair based exercises).

Additionally, the HCPs felt that an exercise intervention that could be extended (in terms of time) would be beneficial, as patients have commented previously that they felt 'kicked out' of previous interventions after a specified number of weeks, where there was no possibility of extending the timescale:

> *"I've done a lot of these group work sessions with patient across the hospital and everyone says that they go along to these things for twelve weeks and then they feel like they're kicked out and they've got nowhere to go after that"*

Overall, clinicians emphasised the need for a flexible intervention, both in terms of content and mode of delivery. The participation of the clinicians and health care professionals was essential in terms of informing the type of exercise to be considered, such as a floor based as well as mode and frequency of delivery.

This led to the idea that this could help build rapport between the patients and their companions that could facilitate social support:

> *"It builds up that whole rapport, doesn't it, I think, that you're helping someone else, the buddy system"*

Patients should be able to do the exercises anywhere

Clinicians suggested that an intervention that could be taken to different locations would be beneficial, for example- an intervention that could be used at home and at a community centre:

> *"I think the local council could support in leisure centres" and "you could maybe take what's given to you to a leisure centre and do it there"*

> *"You can do this at home if you wanted or you could go to a gym to do it supervised and we could get you in to do that. Then that gives the individual choice about whether they want to do it at home, whether they want to do it in the community"*

This suggestion further emphasised the need for a versatile intervention, that could be tailored to individual needs, with the understanding that some patients may not be as fit or as motivated as others. For example, some patients may benefit more from visiting a community leisure centre where additional help and support is available.

Difficulty accessing a central location to attend a training session could be a barrier to uptake

Clinicians did not think it would be feasible to see every patient weekly to monitor their progress with the intervention or to host the vast majority of the intervention at a central location, such as the hospital. They highlighted that the patients often came from a wide geographical area and it wouldn't be feasible for them to attend hospital weekly due to large travel commitments:

> *"That's what we've got to think about because we are a tertiary referral centre, we go all the way to Whitehaven, all the way up to Berwick and all the way down past Middlesbrough and Northallerton… It's a lot to commit to"*

However, they felt that a single training session would be useful to host at a central location, to ensure everyone could attend to provide demonstrations.

6.4.2.1.3. Conclusions of exploratory work

The findings of the exploratory work (i.e. the cohort analysis) highlighted a physiological need to improve preoperative fitness, and the initial focus group discussion provided evidence that patients were interested in taking part in an exercise intervention while on the waiting list. Furthermore, the focus group was important for informing the mode of delivery and specific intervention content (e.g., materials required), that patients felt were necessary to enable them to engage. They emphasised the need for exercise demonstrations and support to acquire the skills to complete them independently. As such, an initial training session was considered important to familiarise patients with the range of exercises offered, their purpose, and how to conduct them safely. Patients reported doubting their own ability to

recall the exact exercises and requested that information was made available throughout the intervention with prompts about the different exercises to perform.

The primary motivation reported by patients for wanting to take part was the opportunity to complete an exercise intervention that was specifically tailored towards them as individuals, and advocated by clinicians. This was primarily for safety reasons, as all patients reported a fear of falling or injuring themselves, possibly while exercising alone, and as such unable to ask for help. This finding was important for highlighting a need to address poor stability, and for the development of an intervention that was diverse and capable of addressing a wide range of symptomatic patients.

The findings of the focus group also highlighted high levels of self-reported depression and anxiety in patients assessed for eligibility for wait-listing, potentially meaning motivation and adherence could be a challenge when compared with 'healthy' populations. Patients raised the issue of being anxious about exercising, particularly when this is not something they regularly engage in. As such it was considered vitally important to develop a rapport with patients to build a trusting relationship, whilst maintaining a supportive, encouraging and friendly environment. Although, this also presents an area where patient education may be lacking. Patients should be encouraged to remain as active as possible as this has consistently been shown to improve symptoms of depression (Mead et al., 2008, North et al., 1990, Craft and Landers, 1998, De Moor et al., 2006).

6.4.2.2. Stage 2: second focus group

The preliminary focus group was essential for exploring and subsequently confirming patients' motivations for taking part in an exercise intervention. However, a greater understanding of the barriers and enabling factors was required to develop an intervention that would appeal to, and benefit, a diverse range of patients. Additionally, further information was required from patients about the type of exercises they felt capable of completing.

To explore this further, a second, more in-depth focus group discussion was undertaken. The aim of this focus group was to explore patients' experiences and attitudes towards community based/home based exercise interventions, training sessions and the role of

technology, the use of exercise diaries and goal setting, with the overall aim to understand how to maximise uptake, engagement and adherence for maximal benefit.

6.4.2.2.1. Second focus group

A purposive sample of participants were invited to attend a focus group discussion to explore preconceived barriers and facilitators towards uptake, adherence and engagement with the intervention and associated materials and suggestions for delivery. Participants included a transplant coordinator, a LiverNorth representative, an anaesthetist, two health psychologists, four patients (one who was waitlisted and three who were transplant recipients) and the partner of a patient. The focus group took place at the Freeman Hospital, Newcastle upon Tyne.

A topic guide (appendix M) was used to guide discussion and prompts to explore confidence and safety around performing the exercises. The views of healthcare professionals and the LiverNorth representative were also sought to understand any barriers and enablers to delivery of the intervention from a clinical perspective. The timescale of the intervention was also discussed, and the use of technology to support the intervention (e.g., for self-monitoring of exercise, information provision etc). The focus group was audio recorded and transcribed verbatim.

Characteristics of focus group participants

Patients were approached to take part in the focus group discussion sampled in terms of where they were in the transplantation process (e.g., listed, transplanted). Four patients attended, three of which were male, age range of 50-62 years. One patient was on the active waiting list at the time of the focus group, and the other three had previously received a liver transplant. Of those who had received a liver transplant, two had their surgery less than 10 years ago, and the other >20 years ago. One patient was accompanied by their partner.

Members of staff in attendance included a transplant coordinator, two health psychologists, a consultant anaesthetist and a representative from LiverNorth. Three members of staff were female and two male.

6.4.2.2.2. Key findings of the second focus group

Following thematic analyses of the data obtained from the second focus group, four themes and one sub-theme were generated, as described below.

Table 6.3. Themes derived from the second focus group

Theme/ sub-theme	**Supporting quotes**
A detailed description of the exercise intervention is required to allow patients to make an informed decision about taking part	*"It depends what kind of exercise you're talking about"* *"How long you want to do it - Is it ten minutes, fifteen minutes a day?"* *"What to expect afterwards"*
Safety procedures act as motivators to take part in an exercise intervention *A guided training session would act as a motivator to uptake and adherence*	*"I think what I've seen with the encephalopathic group... We've got to be very careful about how we do it. Now, that doesn't mean we can't do it at home but maybe they need to do more floor based exercise so we're not going to have them falling over"* *"We have to be careful. I think... put mats around, one the floor, someone with us, that's what I was [thinking]"* *"I'd want to be shown [the exercises] in person I would say"*

	"It's very difficult I think from videos and from paper diagrams to work out [what to do]" *"If you do it in person as well with somebody there, you can actually see whether you can do it"* *"Yes, a physio could show us exercises initially. I mean who better"*
Social and financial support would act as a facilitator to adherence	*"A telephone call to see how you're getting on, if you've got any concerns or issues or just to tell you how wonderful you've been at doing these exercises… just give me more please because it's the best thing since sliced bread."* *"I think the local council could support in leisure centres… So I don't have to join and pay £40 a month or something."*
Setting goals/targets and establishing coping strategies would motivate patients to adhere to the intervention	*"Someone may not be feeling great in one week and then think, "I can't go this week. I'll see how I feel next week," but that's okay too. You've just got to prepare for things like that and prepare the system if you like, the service for something like that. Then it's doable… You know you're still welcome to go back the following week"*

A detailed description of the exercise intervention is required to allow patients to make an informed decision about taking part

When asked about what kind of information would be required to motivate patients to engage with the intervention, responses were linked to the type of exercise to be undertaken, expected amount of exercise to derive benefits to fitness post-transplant recovery, and potential implications on their liver disease, pre-transplant health and wellbeing:

> *"Is it something you do yourself or is somebody going to come to the house and assist you with it?"*

Safety procedures act as motivators to take part in an exercise intervention

None of the patients taking part in the focus groups had participated in any sort of exercise intervention before, however the most important information, unanimously amongst all patients was that regarding their safety.

> *"So safety advice, put mats around"*

> *"You would have to be supervised to be doing the exercise. So that would mean that a family member would have to spend time with them whilst they were doing that but there's no reason why the family member couldn't join in"*

A guided training session would act as a motivator to uptake an adherence

With safety in mind and increasing the likelihood of adherence, patients were motivated by the prospect of an initial guided group-based training session. Patients reported that they would feel more comfortable performing the required exercises away from a clinical setting following a guided training session where they could be shown how to perform exercises and given information on how to perform the exercises safely and to a satisfactory level for maximal benefit.

> *"Yes, a physio could show us exercises initially. I mean, who better?"*.

All patients in attendance agreed that they would feel most comfortable exercising with a partner, using a 'buddy system' or completing exercises in a community setting:

"You could be maybe training with somebody else who's had the same operation, like a liver transplant".

"You can work with each other, help each other, maybe get a bit fitter".

While patients agreed that they would prefer to have group-based sessions weekly within a clinical setting, they felt that a significant travelling commitment would be difficult to maintain for the duration of the intervention. Patients responded positively to the idea of a flexible programme that they could take with them to a local leisure or community centre, but were also keen to be able to exercise at home if required.

Social and financial support would act as a facilitator to adherence

The possibility of family members joining in with the exercises was discussed and considered to be a facilitator to adherence. The LiverNorth representative reported that some community/leisure centres offered discounted membership rates for post-transplant patients to exercise – the same offer may be available to pre-transplant patients, thereby providing an inexpensive means of exercising in the community.

When asked about the type of support that would facilitate adherence and uptake to the intervention, patients suggested that psychological and financial support to facilitate potential travel costs may be needed, as well as the provision of suitable equipment.

"Ranging from psychological to even the [provision of] equipment" and *"financial"*

Setting goals/targets and establishing coping strategies would motivate patients to adhere to the intervention

Focus group participants reported being motivated by monitoring progression or having personalised targets. They also reported an interest in a second CPET upon completion of the intervention.

"You quite like the idea of having almost a start and an end to see how far you've come"

Patients were open minded and flexible about the duration of the intervention, but emphasised a greater need for flexibility in terms of how much exercise would be expected per week, and the need for potential adjustments depending on their liver related health.

> *"That could be part of the coping plan, if I'm not feeling well then I'll do extra next week or something like that"*

In general, setting goals and monitoring progress by keeping an exercise diary was suggested as a suitable means for maintaining compliance and adherence. Patients were keen to use technology to do this. The majority of patients in attendance reported already using technology to monitor their physical activity (i.e. step count per day) using pedometers or activity trackers:

> *"On the health app on my phone, it keeps your blood pressure, your heart rate or whatever. If this could be modified to say keep an eye on you, more specific needs or whatever, keeping an eye on you to say something's wrong here, your blood pressure has changed… then you could keep track of me"*

6.4.2.2.3. Conclusions from the second focus group

The aim of the second focus group discussion was to identify barriers and enabling factors to uptake and adherence to an exercise intervention and to better understand how to make the intervention accessible and feasible to deliver. The findings were essential in order to shape the intervention in terms of content and mode of delivery. Flexibility of the intervention was one of the most salient findings in terms of location and content of the intervention. For example, patients reported to be motivated to complete an exercise intervention at home and/or at a community leisure centre, following an initial guided training session to demonstrate exercises to ensure safety when performing the exercises independently. This approach was reported by patients to increase the likelihood of adherence to the intervention. Patients commented on the differences between themselves and others in terms of fitness, while on the waiting list. As such, they highlighted the need for an intervention that can be individualised depending on each person's needs. Therefore the need for a diverse intervention was emphasised, with possible regressions and progressions available for each patient. Patients were keen to adhere to the intervention for maximal

benefit and therefore reported that goal setting and inclusion of exercise diaries would be beneficial to monitor themselves throughout the intervention.

6.4.2.2.4. Development of alpha prototype intervention

The findings from the second focus group provided an initial indication of the form and content of the alpha prototype intervention. In terms of content, the following section describes the agreed elements to be included, the proposed pathway of the intervention and the proposed mode of delivery. While the immediate goal of the intervention was to improve cardiorespiratory fitness in order improve post-operative prognosis and reduce perioperative risk, exercises to improve strength and balance were also included. The reasons for this were largely to increase patient uptake and engagement, with the hopes of offering a more personalised intervention more in line with patient preference. While the inclusion of balance related exercises, for example, likely won't directly improve cardiorespiratory fitness, it was hoped that the inclusion of exercises that might be more appealing or tolerable to patients would serve to increase prolonged engagement. Accordingly, it was therefore hoped that good engagement with the intervention might serve to increase the PA of patients which has health benefits in itself (previously discussed in Chapters 1 and 5). In order to ensure that patients engage in the more 'beneficial' exercises to improve post-operative prognosis and reduce perioperative risk (cardiorespiratory), the amount of cardiorespiratory exercises must outweigh the prescribed strength and balance related exercises. Overall, it was hoped that a more personalised intervention would serve to increase initial and prolonged engagement, and might potentially serve to give patients more confidence to try other exercises which they might not have previously considered, such as more cardiorespiratory based exercises.

Exercises

Exercises were categorised into cardiorespiratory, strength and balance exercises, namely 'stamina, strength and stability' for presentation to patients and can be seen in greater detail in appendix N. However, briefly, these were:

- Cardiorespiratory exercises.

Results from the cohort analysis (section 6.2) indicated a relatively low level of cardiorespiratory fitness (when defined using anaerobic threshold and VO_{2peak}) and therefore it was considered of significant important to have elements of the intervention that could

help increase, or prevent a decline, in cardiorespiratory fitness levels. Furthermore, given that modern literature (Prentis et al., 2012) strongly associates cardiorespiratory fitness levels as an indicator of post-operative survival, this further corroborated the need for a cardiorespiratory element. Within the focus groups, a salient theme for motivation to adhere was the utilisation of goals or the potential use of a 'before and after' measurement of fitness and strength. Patients further suggested that they would be keen to increase their current levels of fitness and subsequently would like a cardiorespiratory element within the exercise intervention.

- Strength (body weight based exercises or exercises using very light weights, such as tins of beans or small water bottles) exercises.

While no global measures of strength were reported within the cohort analysis, pre-transplant cohorts typically have a large proportion of patients with sarcopenia. Our cohort presented with reduced grip strength indicative of an increased risk of all-cause death, cardiovascular death, cardiovascular disease (Leong et al., 2015), increased severity of liver disease (Johnson et al., 2013) and functional decline (Lai et al., 2016). Therefore, given the strong association between sarcopenia, reduction in grip strength and post liver transplantation mortality, it was considered to be of great importance to incorporate an element of resistance exercise or 'strength' exercises (Englesbe et al., 2010) in order to reduce excessive muscle wasting (Clark et al., 2016). Furthermore, thematic analysis of data derived from the focus groups suggested that patients would like a flexible intervention that could be undertaken in most settings. Resistance exercises are easily modified to not be dependent on expensive or difficult to access equipment. Additionally, for patients who reported concerns about potential lack of safety due to a fall risk, resistance exercises are largely adapTable to chair-based positions, thereby reducing this risk.

- Balance exercises.

Data derived from the focus groups suggested that patients may consider themselves to be at a greater fall risk compared to a 'healthy' population. HCPs in attendance at the focus groups further corroborated that this group of patients are likely to be less stable, and as such be at a greater fall risk. Therefore, an element of stability and balance exercises were considered to be an important aspect of the exercise intervention. Balance exercises are also

easily modifiable to be undertaken in most settings, and are largely not dependent on the availability of specialised equipment. Furthermore, balance/stability training has been shown to be beneficial and safe in patients awaiting solid organ transplantation (Mathur et al., 2009). Within the focus groups, patients reported being limited when attempting to complete activities of daily living due to limitations in their balance and stability, and improvements in the ability to complete activities of daily living were reported as being a potential goal as an outcome from the exercise intervention.

Pathway of intervention and mode of delivery

In order to meet the criteria of a 'personalised intervention', following an initial consultation with the patients and evaluation of the baseline physiological needs, a range of exercises from all three categories would be available for the patient to partake in, individualised to meet their needs and goals. As findings from the focus groups suggested, during the initial consultation, specific, measurable, achievable, relevant and time-based (SMART) goals would be set following discussion between the patient and the member of staff supervising the exercise intervention, in order to devise manageable and motivational goals. Following feedback from the focus groups, in order to facilitate uptake to the exercise intervention, a single guided training session is proposed. At this training session the patient would be shown how to safely perform the relevant exercises and would be given the opportunity to record the instruction for themselves, which could be used as a resource throughout the intervention. Additionally, paper-based resources with information on each exercise would be provided. Patients would be given paper diaries, or the option of completing them on a computer and emailing them to a member of the research team, to allow for monitoring progress and receipt of feedback. Weekly telephone calls were included to maximise compliance and adherence, and to allow monitored progressions or regressions of exercises to be made at timely intervals throughout the intervention.

The specific intervention was further refined upon completion of a third focus group, where the alpha intervention was presented to participants to allow for further feedback in stage 3 of the development process.

6.4.2.3. Stage 3: presentation of alpha prototype intervention

The third, and final focus group discussion was conducted to present the alpha prototype intervention to patients from focus groups one and two and to obtain feedback. All participants were given a paper handbook depicting the intervention and other patient facing materials that would be provided at commencement of the intervention (appendix N). As an adjunct, all participants were given the opportunity to have the planned outline of the intervention delivered to them with supplementary video demonstrations of the proposed exercises. Participant characteristics are described below.

Upon presentation of the alpha prototype, patients were satisfied that their views and suggestions provided during focus groups one and two had been incorporated in to the intervention. Minimal changes were recommended by participants (i.e., patients and clinicians). Suggested changes included the need to incorporate an online element where patients could access videos of exercises being performed correctly, with regressions and progressions available and to have the option of engaging in group exercise classes, despite this being a home-based exercise programme. Patients indicated that online classes could be a useful component if available.

Given the largely positive feedback from the third focus group, the intervention was subject to small refinements and was considered to be developed in line with patient needs, preferences, capabilities and interests.

Characteristics of attendees of the final focus group

The same purposive sample of patients from the previous focus groups were invited to attend the final focus group, based on where they were on the transplantation process. Three patients attended, all of whom were male, within an age range of 52-62 years. One patient was on the active waiting list at the time of the focus group, another had previously received a liver transplant and the other was in process of being assessed for suitability for the transplantation waiting list. The patient who had received a liver transplant had received >20 years ago.

Members of staff in attendance at the focus group comprised of two health psychologists, a consultant anaesthetist and a psychology undergraduate student. Of the members of staff in attendance, there were three females and one male, within an age range of 21-45 years.

6.4.3. The intervention development process

The exercise intervention was developed as a result of the findings from three focus group discussions and informed by the cohort analysis. The intervention delivery plan is summarised in Figure 6.4.

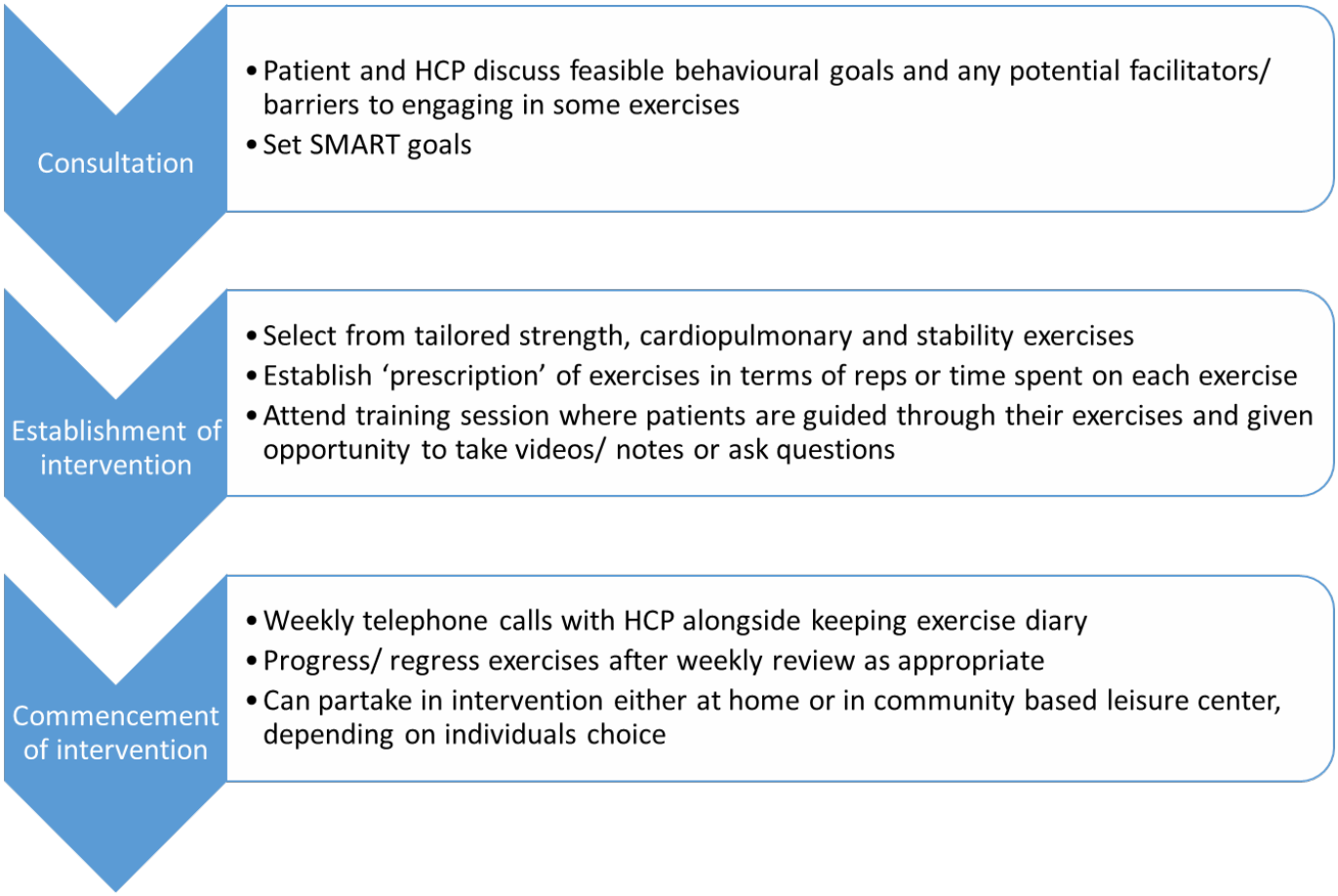


Figure 6.4. Brief outline of developed intervention

6.4.3.1. Consultation

The initial phase of the intervention would involve a primary consultation between patients, any invited friends/family members and the member of staff leading the intervention. The aim of the consultation is to discuss, establish and set SMART goals to work towards throughout the intervention, as well as discuss practical arrangements for taking part. For example, where the patient might feel most comfortable undertaking the exercises, potential space limitations, friends and family who may assist and barriers including time or work constraints.

6.4.3.2. Establishment of intervention

As described in the above flow chart, the establishment of the intervention comprises of the 'prescription' of the exercises, in terms of exercises to be completed, reps performed or time spent engaging in the exercise. Following on from the consultation, and taking in to account practical limitations, current levels of fitness and patient goals, a range of strength, cardiopulmonary and stability related exercises are discussed with the patient. The retrospective cohort analysis described a cohort of patients with a wide range of comorbidities to be accommodated, and highlighted the inability of some patients to undertake the CPET, and highlighted the high fall risk of this population. Furthermore, the prevalence of liver disease related complications (varices, ascites and encephalopathy) further corroborate findings from the focus groups in terms of the need for modified exercises to individualise the exercise intervention to be accessible to the vast majority of patients. The member of staff leading the intervention and the patient then decide which exercises are suitable and a schedule of how many repetitions and how many training sessions each week is agreed. SMART goals are used in order to devise realistic, manageable and measurable targets for the patient.

Specific exercise prescription would be informed largely by the CPET, where applicable, and data generated as part of the transplant assessment process. For example, VO_{2peak} and AT would be used to inform each individuals' potentially maximum exercise and functional capacity. Further exercise prescription would be informed in line with the exercise prescription guidelines as recommended by the American College of Sports Medicine (ACSM) (Swain et al., 2014). For example, for cardiorespiratory exercises, exercises would aim to work at between 60-70% of the patients maximum VO_{2peak} as determined by the initial CPET. To make the intervention widely applicable to all patients, without the need to invest in and educate on the use of heart rate monitors, rate of perceived exertion (RPE) would be used as a surrogate of exercise intensity (Shigematsu et al., 2004). Furthermore, the conversion of VO_2 to the metabolic equivalent of task (MET), whereby 1 MET is the approximate equivalent to 3.5 ml/kg/min, would be used to inform exercise intensity. To give a specific example, if a patient had achieved a VO_{2peak} of 15 ml/kg/min at their transplant assessment CPET, 60-70% of this would indicate a target VO_{2peak} of 9-10.5 ml/kg/min, which is the equivalent to 2.6-3 METs. This would then correspond to a slow-moderately paced walk or slow stair climbing.

The frequency and time spent engaging in the exercise would be titrated to each individuals needs over the first week or so of the intervention, and specific clinical parameters (such as the presence of ascites, varices or history of hepatic encephalopathy) would inform the specific type of exercise that might be suitable for each individuals. Specifically, someone with hepatic encephalopathy may be restricted to chair based exercises to minimise fall risk, while those with the presence of ascites or varices may be restricted from doing exercises where they must lay down or have specific engagement through their abdominal muscles, in order to reduce discomfort and maximise exercise tolerability (Tandon et al., 2018). If patients had not been able to partake in the CPET, they would be given an opportunity to experiment with various exercises under supervision in order to better understand their exercise capacity to inform the exercise prescription.

Subsequently, patients have the option to attend a guided training session, either in person or online using video call software. The purpose of this session would be to demonstrate the correct way to perform the exercises, where the patient is given the opportunity to try the exercises and receive feedback. The patient would also be able to take notes, record any useful footage and discuss potential progressions and regressions of the exercises.

6.4.3.3. Commencement of intervention

Following the initial training session, patients would have support available, in the form of weekly telephone calls from a member of staff running the exercise intervention. Consenting patients would be grouped into support groups, where they could support one another, in terms of providing motivation to adhere to the exercise intervention, or simply provide emotional support throughout the wait-listing procedure. During weekly reviews, patients are given the option of progressing or regressing their exercises. Additionally, patients are encouraged to maintain an exercise diary, in order to keep track of their progress and facilitate feedback provision during telephone calls.

While patients' baseline CPET would serve as the primary entry assessment, it is anticipated that there will be no formal exit assessments of the intervention as it is likely patients will likely finish partaking in the intervention due to receiving a liver transplant. Indeed, other prehabilitation studies in people awaiting liver transplantation have experienced a large portion of people unable to attend or complete exit assessments due to the availability of a donor liver at short notice (Williams et al., 2019). However, patients were keen to potentially

partake in another CPET, and HCPs in attendance at the focus group supported the idea of scheduling another CPET at 12 weeks from starting the programme. Therefore, it is anticipated that another CPET would be scheduled at 12 weeks, and this could feed into clinical care to provide an updated report of patients functional capacity, however, the variable time spent on the waiting list means that it is probable a large proportion of patients will not be able to attend the second CPET.

Delivery of the intervention

Attendees at the focus groups particularly advocated the use of a multidisciplinary team to deliver the intervention. The transplant coordinator in attendance voiced that their pre-existing workload would not enable them to deliver and manage the intervention, and patients in particular advocated the use of physiotherapists or exercise physiologists to lead the training sessions, establish suitable exercise prescriptions and progress/regress the exercises as required. Importantly, this person delivering the intervention would be the point on contact and enable a rapport to be built between the patient and staff member delivering the intervention. Patients expressed concerns about safety and therefore expressed the need to have someone knowledgeable about exercise and physical activity, but also aware of the limitations imposed by the clinical complications of their liver disease and associated comorbidities. The transplant coordinator and anaesthetist in attendance felt that it would be acceptable within their existing roles to refer people to the programme, and both said they would be motivated to be available to provide any medical guidance or advice required. It is therefore likely that a specialised member of staff would be employed to manage the intervention in order to not place extra demands on the time of existing HCPs and ensure that the intervention was lead with someone whose expertise are in the realm of physiotherapy or exercise physiology.

6.4.3.4. Patient facing materials

Appendix N provides a draft of the booklet patients would receive upon commencing the intervention. This booklet contains sections in which patients can record goals, and make notes from the training workshop. Patients would also receive video recordings of the exercises being performed, which include safety tips, progressions and regressions.

6.4.3.5. *Individualisation of the intervention*

An important topic discussed during the focus groups was the need for personalisation. This was further supported by the retrospective cohort analysis, which indicated a wide range of functional capacities within the target clinical cohort. The range of fitness within the cohort is largely due to the varying indications for transplantation, as well as differing comorbidities, levels of frailty and age. For this reason it was essential to develop an intervention that includes exercises with available progressions/regressions to be appropriate to a range of patients with varying capabilities.

6.4.4. Discussion

6.4.4.1. *Development of the exercise intervention*

The focus group discussions were essential for informing the development of the exercise intervention. They provided information that was essential for informing predictors of uptake (i.e. motivations) and for conceptualising the structure, specific content and mode of delivery. For example, findings further evidenced the need for a flexible, individualised intervention that could be easily adapted to a home-based intervention or an intervention for delivery/completion in a community setting.

While previous exercise programmes have been identified as safe for completion by patients with liver disease, there is limited data reporting on the feasibility, usability and acceptability in a cohort of patients who are on the waiting list (Williams et al., 2019). A published protocol (Williams et al., 2018) reports that patients who have taken part in a home based exercise programme will be invited to take part in a process evaluation focus group that will provide feedback on a series of research questions. However, these data have not been published.

While 18 patients were recruited into the aforementioned feasibility study, only 12 were assessed at 6 weeks and 9 at 12 weeks follow-up. The main reason reported for withdrawal was removal from the waiting list due to a receiving a liver transplant. This means that the study was likely not powered to detect statistical significance when assessing changes in quality of life and functional capacity. However, it does provide further insight into the number of patients that should be a target for recruitment in future studies. Similarly, findings also indicate that patients should be recruited at the soonest possible convenience onto

exercise interventions to increase the likelihood of completion of the intervention in order to fully ascertain the potential benefits of the intervention. This presents a real world challenge where it is likely that many patients would not complete an exercise intervention in a clinical setting due to the unpredictable nature of how long an individual will spend on the waiting list. This supports data obtained during focus group discussions reinforcing that an exercise intervention should be highly individualised and based on patient goals and clinical outcome data. Combined with the data obtained from the Williams *et al.* feasibility study, the data from focus group discussions and the retrospective cohort analysis provides a basis for evaluating the feasibility of delivering the exercise intervention and undertake exploratory analyses on the impact on functional capacity of patients on the liver transplantation waiting list.

Going forward, further work is required. Specifically, establishing the practicality of embedding this intervention into current clinical pathways, assessing the feasibility of recruiting to this programme and assessing the fidelity of delivery. Furthermore, more specific information should be collected to inform the exact nature of staffing needs through more focus groups. A small scale feasibility study would serve to inform about the practicality of delivering the intervention and monitoring engagement and adherence. Should this intervention prove to be feasible to recruit to and show good engagement throughout, this could serve to inform about the staffing needs and pragmatic considerations required to scale this up to a functioning clinical service within the Freeman Hospital.

6.4.4.2. Limitations

There are limitations to this work that should be acknowledged. With regards to the cohort analysis, the data used was sourced from a single centre, with all data recorded from patients assessed at the Freeman Hospital, Newcastle upon Tyne, UK. While this facilitated an in-depth analysis of information, the data collected portrays patients from predominantly the North East of England, although a minority did travel from outside of the North East region. All data were acquired through the analysis of TAM reports, therefore it is plausible that a small proportion of information may have been overlooked. It is difficult to accurately report on patient's pre-transplant lifestyles in the absence of objective measures of lifestyle behaviours such as PA/exercise and dietary intake that would have provided a more accurate insight. Due to the high number of patients unable to start the CPET, the AT scores for each sub-group do

not reflect the full fitness of the assessed cohort of patients and this is a limitation of the study where the mean fitness in the non-listed group is likely much lower than reported.

A primary limitation is the lack of sample diversity within the three focus group discussions that were conducted to obtain the views of patients and HCPs. Patients were purposively sampled based on where they were on the transplant pathway, and therefore not purposively sampled to achieve diversity in terms of age, gender and ethnicity. For example, all patients in attendance were white, only one participant was female and most were of a similar age (50-62 years). Future studies should aim to explore the views of a more representative sample in terms of gender and ethnicity to gain a more representative understanding of potential facilitators and barriers to adherence and motivators to uptake. Similarly, the views of patients from a wider age range should be explored, as well as views from patients with a broader range of fitness and underlying aetiologies/indications for transplantation and more information should be obtained relating the delivery of the intervention. Furthermore, the inclusion of patients from a single centre in the North East of England may confer some geographical bias.

While the intervention developed incorporated the views of patients transplanted, listed for transplant and clinicians responsible for patient care, the acceptability and feasibility of the intervention requires formal assessment to establish whether the it is fit for purpose (i.e. whether it is both acceptable to patients and clinicians and feasible to deliver).

6.4.5. Conclusions

Subsequent to successful listing, the data obtained from the cohort analysis and the focus group discussions indicate that patients who are on the waiting list often have sub-optimal fitness and would benefit from an exercise intervention. Data generated by the focus group discussions suggest that with appropriate information outlining the benefits of exercise, patients would be motivated and willing to take part in a personalised exercise intervention. Importantly, the feedback obtained from HCPs suggests that it could be feasible to deliver this intervention, however, further research is required to explore this further.

7. Chapter 7: General Discussion

7.1. Summary

The research presented in this thesis examined the potential for lifestyle interventions as therapy in liver disease. Specifically, it explored the feasibility and acceptability of an 800kcal/day VLCD to achieve sustained significant weight loss in patients with NAFLD, as well as the development of an exercise programme targeted towards patients on the liver transplantation waiting list. This Chapter will consider and collate the findings of experimental Chapters 3 to 5, the impact of these findings on clinical practice and how this relates to the current literature regarding lifestyle treatments within NAFLD populations. Furthermore this Chapter will consider the impact of the clinical findings in Chapter 6 and how these can be applied to clinical practise. Finally, this Chapter will describe future research directions that this thesis highlights.

7.2. Acceptability and feasibility of a VLCD to achieve significant sustained weight loss in patients with NAFLD

The data presented in Chapter 3 is, to the best of our knowledge, the first of its kind to report on a VLCD in a cohort of patients with NAFLD. While previous research has reported the efficacy of a VLCD intervention within cohorts that incidentally may have had patients with NAFLD (Lean et al., 2019, Jebb et al., 2017), the impact that a VLCD may have specifically on NAFLD had not previously been assessed. Prior to the data presented in Chapter 3, a VLCD had been shown to facilitate significant weight loss in patients with obesity (Jebb et al., 2017) and T2DM (Lean et al., 2019), and this was also associated with the reversal of diabetes in 46% of patients (Lean et al., 2018).

The primary outcomes of our study were to assess the feasibility of recruiting patients with NAFLD to a VLCD and the acceptability of the intervention. Data presented in Chapters 3 and 4 indicate that a VLCD intervention is feasible to undertake and acceptable to patients with NAFLD. Overall, the study was fully recruited from a single centre within 6 months, and only 3 (10%) of patients withdrew during the VLCD intervention. Of those who completed the intervention, 59% achieved >10% weight loss post VLCD, and 34% of the whole cohort maintained ≥10% weight loss for at least 6 months after completing the VLCD intervention

(Scragg et al., 2020). Compared to standard care (Vilar-Gomez et al., 2015), our data presented favourable results. For example, it has been reported that within standard care, 70% of patients achieved <5% weight loss, 20% achieving between 5 and 10% weight loss, and just 10% achieving a weight loss of greater than 10% at 12 months (Vilar-Gomez et al., 2015).

Overall, the observed adherence and subsequent achieved weight losses are consistent with previous studies that have assessed the feasibility of a VLCD in individuals with obesity and T2DM (Lean et al., 2019, Jebb et al., 2017). DiRECT observed a mean reduction in bodyweight of 10 kg at 12 months (IOC analysis), and we observed a mean weight loss of 10 kg at 9 months (IOC). Similarly, retention between the two studies were similar, with DiRECT and ourselves reported 10% of patients withdrawing throughout the initial VLCD intervention. Given the similar protocol employed to achieve a VLCD, variances in results are likely due to the varying baseline cohort; for example, DiRECT had a mean baseline BMI of 35 kg/m^2, whereas ours was 42 kg/m^2 (Lean et al., 2018). The comparable nature of the achieved weight loss and retention is encouraging, as this suggests that the VLCD intervention was as acceptable to individuals with NAFLD as it was to those with T2DM.

Furthermore, the secondary outcome data (specifically, that which relates to improvements in markers of liver and cardiometabolic health) presented in Chapter 3 compares favourably to pharmacological trials (Younossi et al., 2019, Ratziu et al., 2016). For example, while a recent study assessing the impact of vitamin E and obeticholic acid reported similar outcomes with regards to changes in liver enzymes, the use of obeticholic acid was associated with a rise in total and LDL cholesterol levels, alongside a fall in HDL cholesterol (Younossi et al., 2019). The use of obeticholic acid has also been associated with an increased incidence of pruritus; the REGENERATE study reported an incidence of 45% of patients taking a dose of 25 mg/d, compared to 19% within the control group (Malnick et al., 2020, Younossi et al., 2019).

Going forward, it is essential to establish the true efficacy of a VLCD on liver health in a NAFLD population, by assessing this in a larger cohort and directly measuring liver fat, inflammation and fibrosis. To gain a greater understanding of the potential efficacy of a VLCD to improve characteristics of NAFLD, a control group should be included in future studies. For example, a randomised controlled trial with patients randomised to an intervention arm and a standard care arm. While fibroscan and non-invasive score have been associated with disease progression there can be inaccuracies when using these. However, for the benefit of

feasibility, these measures were appropriate as they reflect current clinical practice. Future work should also aim to report weight maintenance over a longer period of time, beyond 9 months. It would be of interest to establish predictors of resolution of fibrosis or other hallmarks of worsening disease progression. For example, analysis of DiRECT data has been undertaken to assess potential predictors of remission of T2DM (Thom et al., 2020b), where it has been reported that weight loss is the major predictor. Early weight loss and programme attendance were also shown to predict a high rate or remission. This correlates well with data presented in Chapter 3 that strongly associated with early weight loss with final weight loss. Additionally, we reported a dramatic initial rise and subsequent fall in liver enzymes (AST and ALT). While there are some hypotheses on the underlying mechanism of this phenomenon, further research is required. This is important as to investigate the potential for initial liver damage following a VLCD to achieve significant weight loss, and it may be that this may be an intervention that is not suitable for some patients due to the potential to cause harm as a result of the observed initial liver damage.

Overall, the results from Chapter 3 indicate that a VLCD is an acceptable and feasible treatment for some patients with NAFLD, and should be further explored in larger sample sizes and over a greater period of time.

7.3. Factors associated with engagement and adherence to a VLCD to achieve significant weight loss in patients with NAFLD

Chapter 3 demonstrated the feasibility and acceptability of a VLCD intervention in patients with NAFLD quantitatively, however, given the perceived drastic nature of the intervention it was important to further explore potential barriers and facilitators to uptake, engagement and adherence. Therefore, immediately post-VLCD intervention, semi-structured interviews were undertaken in order to ascertain patient views and feedback, which would also inform future studies.

Overall, the VLCD intervention was perceived to be acceptable and easier to adhere to than expected. The most salient barrier to adherence was reported to be irregular working hours, while other barriers include employment in physically demanding jobs and family members

providing temptation to deviate from the study protocol. On the other hand, rapid weight loss, support from healthcare professionals during study visits, social support from family and friends outside of study visits and ease of following the VLCD were reported as facilitators to adherence. To overcome perceived barriers, participants employed multiple behavioural regulation strategies including goal setting, planning, avoidance of food and difficult social situations and self-distraction. Feedback and further recommendations obtained from the interviews reported that support from other participants, a report card summarising anthropometric outcomes/markers of metabolic health from each visit to take away with them, and more recipes for the daily vegetable servings would be beneficial to further support adherence.

Previous research had indicated that the motivations for NAFLD patients undertaking a VLCD are likely to be different to those of other cohorts, such as patients with T2DM *(Lean et al., 2019)*, particularly because many patients with NAFLD are asymptomatic and are not concerned about their condition (Avery et al., 2017, Haigh et al., 2019, Hallsworth et al., 2019). This is unsurprising, as it has previously been reported that some patients with NAFLD are not appropriately referred (Hallsworth et al., 2019) and therefore may not receive the correct support or advice relating to their risk of disease progression. Furthermore, awareness of NAFLD is reportedly low, even in populations at the greatest risk (Wieland et al., 2015). However, in our study, qualitative analysis revealed that patients were aware of the risk to their overall health that NAFLD poses, and a primary motivator for patients to take part in the study was to lose weight to benefit their health, rather than for aesthetic reasons. Similarly, within patients who took part in the DiRECT trial, a common motivator to partake was to improve their health, particularly in relation to their complications caused by diabetes (Rehackova et al., 2020). Given that our patients were in the ongoing care of a hepatologist prior to partaking in the study, and willing to partake in a research study, it is likely that this cohort may not be representative of a typical NAFLD cohort. Therefore, it is important to consider that the motivators for these patients are likely not the same as those do not regularly consult with a clinician with regards to their NAFLD. Going forward, it may be informative to attempt to interview a larger pool of eligible patients in order to ascertain preconceived notions regarding the potential participation in a VLCD intervention. Similarly, further studies would benefit from obtaining feedback and information regarding barriers to

engagement and adherence from those for who the intervention was not a success, in order to gain a more representative pool of information.

Data presented in Chapter 4 provides evidence that the VLCD was widely acceptable for a range of individuals and during analysis data saturation was achieved following approximately the 15th interview. Therefore, the themes presented are strongly supported by the data collected. Going forward, in order to increasing the likelihood of representing more individuals with NAFLD, further interviews should take place in individuals with different cultural and ethnic backgrounds. As with the qualitative data obtained in the DiRECT, the views of younger patients were not sufficiently represented (Rehackova et al., 2020). However, this is likely representative of a typical NAFLD cohort as NAFLD is more prevalent in middle-age and beyond.

Other identified themes in our data have some similarities to qualitative data obtained in other cohorts, such as individuals with obesity, T2DM and polycystic ovarian syndrome (Rehackova et al., 2020, Rehackova et al., 2016, Love et al., 2016, Östberg et al., 2011). For example, the need for social support outside of the parameters of the study visit, highly regimented intervention to facilitate an 'easy to follow" protocol and the importance of clinical advocacy as a primary motivator to uptake (Rehackova et al., 2020, Rehackova et al., 2016, Love et al., 2016, Östberg et al., 2011). On the other hand, our data also presented some themes which had previously not been reported, such as the need for more flexible working arrangements in order to not be excessively fatigued while undertaking the intervention, as this led to the desire for quick and convenient satiating food which were not within the parameters of the protocol. As an extension of this, this also presented the need for the intervention potentially needing to be discussed with work superiors, to facilitate time off in order to attend the study visits. Given that attendance to study visits was highlighted as being integral in maintaining engagement and adherence, this is of particular importance.

Findings from the study depicted in Chapter 4 provide information on the motivators, facilitators and barriers to engagement and adherence with regards to a VLCD, and adds valuable insight into the current qualitative evidence surrounding both VLCDs and the attitudes towards changing lifestyle behaviours in individuals with NAFLD. Providers of a VLCD intervention, both clinical and commercial, would benefit from using these findings to support individuals attempting to adhere to a VLCD. For example, these findings inform that sufficient

guidance should be provided, and that planning in advance is a useful behavioural methodology to help individuals overcome barriers. Future studies could provide more information to the pool of current evidence by assessing whether the reported themes are relevant beyond weight loss, throughout weight maintenance.

7.4. Objectively measured physical activity and sleep data in a NAFLD cohort

Chapters 3 and 4 provide evidence of the feasibility and acceptability of a VLCD in a NAFLD population. Chapter 5 further supports the importance of lifestyle behaviours within a NAFLD cohort by describing the differing physical activity (PA) behaviours between our NAFLD cohort and age- and gender-matched controls. Furthermore, Chapter 5 also describes a brief exploratory analysis on the effect of significant weight loss on the PA of our cohort.

Chapter 5 presents data which describes a trend of lower levels of all-intensity PA, higher levels of inactive behaviours and poorer sleep quality and shorter sleep duration in our NAFLD cohort compared to healthy controls. This data was novel in using triaxial acceleromtery to assess PA data between individuals with clinically confirmed NAFLD and healthy controls; previous research has described this using biaxial accelerometry (Hallsworth et al., 2015). Similarly, previous research has described differences between the PA of NAFLD and healthy populations using triaxial accelerometery (Gerber et al., 2012), where the NAFLD cohort were defined using the fatty liver index (which is predominantly determined using BMI) and not a clinical diagnosis.

Given the strong association between the pathophysiology of obesity and NAFLD, it was not possible to match the BMI between our NAFLD cohort and the healthy controls. Some of our patients had extremely high BMI scores and it would be very unlikely that we recruit a healthy control, free of any comorbidities, with similar BMI scores. Therefore, an exploratory analysis was undertaken where BMI was controlled for between the groups. Results from this analysis indicate that BMI is likely a key influencer of PA, however, the data still describes significant differences in levels of moderate PA and MVPA between groups and further justifies the

important role that patients with NAFLD should be counselled on the benefits that increasing the levels of PA can have. This also further supports previous evidence that exercise can alleviate some of the physiological burden of NAFLD independent of weight loss (Hallsworth et al., 2011).

Our data reported no significant changes in PA, inactivity or sleep between baseline, post-VLCD and follow-up. This is similar to data reported by Lean *et al* within the DiRECT study (Lean et al., 2018). Furthermore, reported PA, inactivity and sleep data were comparable between DiRECT and our study. Potentially, it is likely that no changes were observed throughout the intervention as there was not a particular focus on PA. In our study, as described in Chapter 3, some patients were referred to 'exercise on referral' schemes during the weight maintenance period, but approximately half of the referred individuals had not been inducted into the scheme by the time that follow-up measurements were taken. In addition, the relatively small sample size that had datasets at all time points may not be large enough to reflect any observed changes.

Therefore, future studies should aim to assess potential changes in PA, inactivity and sleep in a larger sample size, and over a longer follow-up duration. Given that NAFLD is directly associated with lower levels of PA and exercise, the data presented in Chapter 5 further supports the recommendations that all patients with NAFLD should be encouraged to increase their PA levels and reduce sedentary time where possible.

7.5. The development of an exercise programme for patients waitlisted for liver transplantation

While Chapter 5 provides evidence for the importance role that PA can have in patients NAFLD, Chapter 6 focuses on the role of exercise following significant disease progression into end-stage liver disease in those awaiting liver transplantation. Figure 7.1 briefly outlines the potential pathway of NAFLD progression through to cirrhotic complications and the need for liver transplantation with a focus on the important potential roles for lifestyle behaviour as therapy. However, this schematic represents solely the individuals who may require a liver transplantation due to NAFLD, whereas Chapter 6 focused on all causes of end-stage liver

disease and therefore encompasses a range of underlying indications for liver transplantation. Over the past decade, NAFLD as an indication for liver transplantation has increased at a rate that far exceeds all other indicators (Wong et al., 2015, NHS, 2019) and therefore the outlined pathway depicted in Figure 7.1 is becoming more and more of a reality.

Chapter 6 outlines the development of an exercise programme targeted at patients on the waiting list for a liver transplant. The exercises developed were largely informed by three focus groups, undertaken with a range of patients at different points in regards to their liver transplantation; some were on the waiting list, and some were post-transplant. Relevant members of staff, who would likely play a role in the delivery of the intervention, were included, to ensure the exercise programme was developed with pragmatism in mind. To provide a quantitative rationale for the need for an exercise programme, and to provide an insight in functional capacity of the potential recipients of the programme, a retrospective cohort analysis was undertaken. This highlighted that lack of fitness or the need to make lifestyle changes was a primary reason for ineligibility for transplantation. This data is in line with previous research which has identified fitness, defined in this instance by anaerobic threshold, an important predictor of post-transplant 90 day survival (Prentis et al., 2012). Furthermore, this further supports the overarching theme of this thesis; lifestyle behaviours are applicable interventional therapies within liver disease across the disease spectrum.

The results of the cohort analysis, combined with previous research emphasising the importance of perioperative fitness, provided sufficient evidence that an exercise programme targeting patients on the waiting list was needed. Within the UK, the median time spent on the waiting list across all transplantation centres was 99 days (NHS, 2019). This provides an opportune time period for patients to engage in prehabilitative therapies. Therefore, the programme was developed in line with patient and health care professional feedback provided at each focus group. The inclusion of patients in the conception of the programme was essential for establishing preconceived barriers and facilitators to uptake, engagement and adherence. Furthermore, this alongside the quantitative data from the cohort analysis, provided a benchmark of the level of exercise intensity that would be feasible for patients to undertake. The inclusion of patients who had previously been a recipient of a liver transplant and who had experienced being on the waiting list further confirmed that an exercise

programme would have been well received and that time spent on the waiting list would be an opportune time to partake.

Assessing the feasibility and acceptability of the intervention was beyond the scope of this thesis. Next steps would be to run the exercise programme alongside a series of focus groups or interviews to obtain feedback from both the staff involved in the delivery of the intervention, and the patients with the goal of increasing acceptability. Additionally, it would be informative to investigate the adherence and efficacy of differing modes of delivery- as described in Chapter 6, a highly flexible programme was developed, giving patients the option of undertaking the intervention at home or within a community setting, with online support available. Similarly, should the programme prove to be efficacious, it would important to assess the cost effectiveness, as well as the overall impact on the post-operative outcomes, in order to determine the potential benefit this could have to current clinical practise.

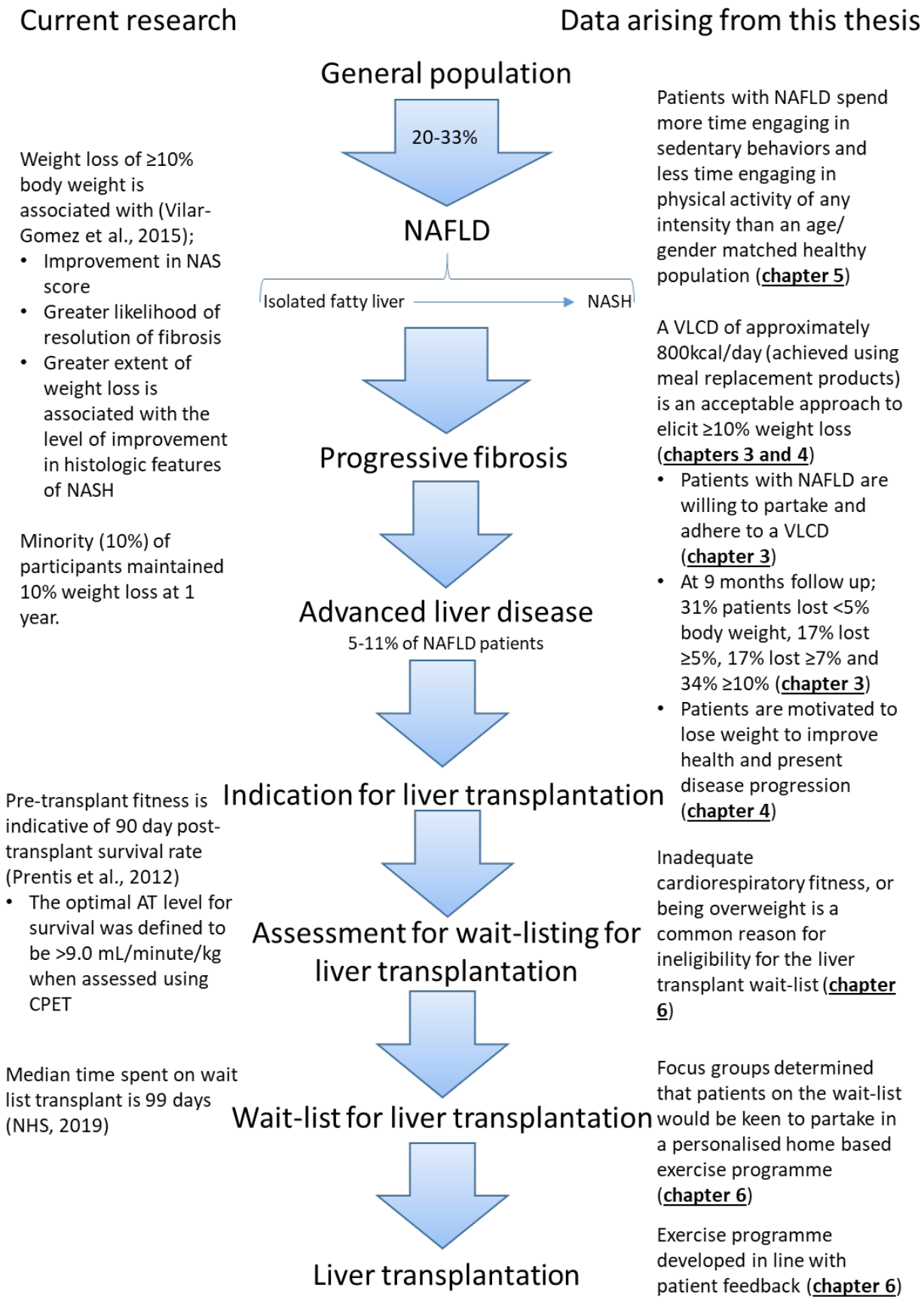


Figure 7.1. Schematic outlining the interlinking pathway between the data presenting in chapters 3-6, and briefly outlining the relevance of the generated data in the context of current clinical practise and literature

7.6. Limitations of the studies presented within this thesis

Specific limitations for each study are addressed in the respective experimental Chapters. The findings reported from this thesis are largely based small scale feasibility studies, and as such, further evidence is required before these findings can be considered part of the accepted evidence base. Research in this area, particularly in the findings reported in Chapter 3, would benefit from a longer follow-up period and a larger cohort to be adequately powered to ascertain truly significant findings with regards to the effectiveness of the intervention. Furthermore, should the intervention prove to be efficacious, it would be essential to assess uptake into clinical practice and the acceptability of delivery to health care professionals.

Another significant limitation within the data presented throughout this thesis is the lack of ethnic diversity. While our data provides insight into the feasibility, potential facilitators and barriers to adherence and facilitators of typically 'hard to reach' populations with regards to adhering to a VLCD, it is limited in reflecting the views of only Caucasian individuals. Similarly, within Chapter 6, the focus groups explored patient feedback from only Caucasian individuals and therefore may not be representative of a large portion of the target population.

Another limitation throughout this thesis is that of the strong potential for volunteer bias; patients who are willing to attend focus groups are more likely to be motivated to take part in an intervention and therefore the obtained feedback may not represent the views of those who are less motivated.

7.7. Conclusions

The findings of this thesis demonstrate the potentially important role that lifestyle interventions could have in managing liver disease and on preventing disease progression in patients with NAFLD. Specifically, these preliminary findings suggest that lifestyle interventions such a VLCD are likely acceptable to many patients with NAFLD and are feasible to recruit to and deliver. These findings also suggest that patients with end stage liver disease might still be motivated to partake in physical activity and exercise based lifestyle interventions. Overall, this thesis presents that lifestyle interventions could potentially be suitably administered as treatment for liver disease and may pave the way for further, larger

scale interventions to be trialled. Should the findings of further, larger studies be in keeping with the data presented in this thesis, lifestyle interventions could become part of recommendations for treatment within standard care.

References

AURORA: Phase 3 Study for the Efficacy and Safety of CVC for the Treatment of Liver Fibrosis in Adults With NASH: NCT03028740.

EASL 2013;EASD 2013;EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Journal of Hepatology,* 64**,** 1388-1402.

Phase 3 Study to Evaluate the Efficacy and Safety of Elafibranor Versus Placebo in Patients With Nonalcoholic Steatohepatitis (NASH) (RESOLVE-IT): NCT02704403.

Study Evaluating the Efficacy and Safety of Obeticholic Acid in Subjects With Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis (REVERSE): NCT03439254.

ABENAVOLI, L., BOCCUTO, L., FEDERICO, A., DALLIO, M., LOGUERCIO, C., DI RENZO, L. & DE LORENZO, A. 2019. Diet and non-alcoholic fatty liver disease: the Mediterranean way. *International journal of environmental research and public health,* 16**,** 3011.

ABENAVOLI, L., GRECO, M., MILIC, N., ACCATTATO, F., FOTI, D., GULLETTA, E. & LUZZA, F. 2017. Effect of Mediterranean diet and antioxidant formulation in non-alcoholic fatty liver disease: a randomized study. *Nutrients,* 9**,** 870.

ADUEN, J. F., SUJAY, B., DICKSON, R. C., HECKMAN, M. G., HEWITT, W. R., STAPELFELDT, W. H., STEERS, J. L., HARNOIS, D. M. & KRAMER, D. J. 2009. Outcomes After Liver Transplant in Patients Aged 70 Years or Older Compared With Those Younger Than 60 Years. *Mayo Clinic Proceedings,* 84**,** 973-978.

AFSHAR, S., SEYMOUR, K., KELLY, S. B., WOODCOCK, S., VAN HEES, V. T. & MATHERS, J. C. 2017. Changes in physical activity after bariatric surgery: using objective and self-reported measures. *Surgery for Obesity and Related Diseases,* 13**,** 474-483.

ALEMANY-PAGÈS, M., MOURA-RAMOS, M., ARAÚJO, S., MACEDO, M. P., RIBEIRO, R. T., RAMALHO-SANTOS, J. & AZUL, A. M. 2020. Insights from qualitative research on NAFLD awareness with a cohort of T2DM patients: time to go public with insulin resistance? *BMC public health,* 20**,** 1-14.

AMATI, F., DUBÉ, J. J., COEN, P. M., STEFANOVIC-RACIC, M., TOLEDO, F. G. & GOODPASTER, B. H. 2009. Physical inactivity and obesity underlie the insulin resistance of aging. *Diabetes care,* 32**,** 1547-1549.

ANDERSEN, R. E., BLAIR, S. N., CHESKIN, L. J. & BARTLETT, S. J. 1997. Encouraging patients to become more physically active: the physician's role. American College of Physicians.

ANGULO, P. 2007. GI epidemiology: nonalcoholic fatty liver disease. *Aliment Pharmacol Ther,* 25**,** 883-9.

ANGULO, P., HUI, J. M., MARCHESINI, G., BUGIANESI, E., GEORGE, J., FARRELL, G. C., ENDERS, F., SAKSENA, S., BURT, A. D., BIDA, J. P., LINDOR, K., SANDERSON, S. O., LENZI, M., ADAMS, L. A., KENCH, J., THERNEAU, T. M. & DAY, C. P. 2007. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology,* 45**,** 846-54.

ANGULO, P., KLEINER, D. E., DAM-LARSEN, S., ADAMS, L. A., BJORNSSON, E. S., CHARATCHAROENWITTHAYA, P., MILLS, P. R., KEACH, J. C., LAFFERTY, H. D., STAHLER, A., HAFLIDADOTTIR, S. & BENDTSEN, F. 2015. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology,* 149**,** 389-97.e10.

ANSTEE, Q. M., NEUSCHWANDER-TETRI, B. A., WONG, V. W.-S., ABDELMALEK, M. F., YOUNOSSI, Z. M., YUAN, J., PECORARO, M. L., SEYEDKAZEMI, S., FISCHER, L. & BEDOSSA, P. 2020. Cenicriviroc for the treatment of liver fibrosis in adults with nonalcoholic steatohepatitis: AURORA Phase 3 study design. *Contemporary clinical trials,* 89**,** 105922.

ANSTEE, Q. M., TARGHER, G. & DAY, C. P. 2013a. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol,* 10**,** 330-44.

ANSTEE, Q. M., TARGHER, G. & DAY, C. P. 2013b. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nature Reviews Gastroenterology &Amp; Hepatology,* 10**,** 330.

ARIAS-LOSTE, M. T., RANCHAL, I., ROMERO-GÓMEZ, M. & CRESPO, J. 2014. Irisin, a link among fatty liver disease, physical inactivity and insulin resistance. *International journal of molecular sciences,* 15**,** 23163-23178.

ARON-WISNEWSKY, J., CLEMENT, K. & PÉPIN, J.-L. 2016. Nonalcoholic fatty liver disease and obstructive sleep apnea. *Metabolism,* 65**,** 1124-1135.

ARULRAJ, R. & NEUBERGER, J. 2011. Liver transplantation: filling the gapbetween supply and demand. *Clinical Medicine,* 11**,** 194-198.

ARYA, A., HERNANDEZ-ALEJANDRO, R., MAROTTA, P., UHANOVA, J. & CHANDOK, N. 2013. Recipient ineligibility after liver transplantation assessment: a single centre experience. *Canadian Journal of Surgery,* 56**,** E39-E43.

ASRANI, S. K. & KIM, W. R. 2010. Organ allocation for chronic liver disease: model for end-stage liver disease and beyond. *Current opinion in gastroenterology,* 26**,** 209-213.

ASTBURY, N. M., ALBURY, C., NOURSE, R. & JEBB, S. A. 2020a. Participant experiences of a low-energy total diet replacement programme: A descriptive qualitative study. *PloS one,* 15**,** e0238645.

ASTBURY, N. M., AVEYARD, P., NICKLESS, A., HOOD, K., CORFIELD, K., LOWE, R. & JEBB, S. A. 2018. Doctor Referral of Overweight People to Low Energy total diet replacement Treatment (DROPLET): pragmatic randomised controlled trial. *BMJ,* 362**,** k3760.

ASTBURY, N. M., TUDOR, K., AVEYARD, P. & JEBB, S. A. 2020b. Heterogeneity in the uptake, attendance, and outcomes in a clinical trial of a total diet replacement weight loss programme. *BMC medicine,* 18**,** 1-9.

AVERY, L., EXLEY, C., MCPHERSON, S., TRENELL, M. I., ANSTEE, Q. M. & HALLSWORTH, K. 2017. Lifestyle behavior change in patients with nonalcoholic fatty liver disease: A qualitative study of clinical practice. *Clinical Gastroenterology and Hepatology,* 15**,** 1968-1971.

AZEVEDO, M. R., ARAÚJO, C. L. P., REICHERT, F. F., SIQUEIRA, F. V., DA SILVA, M. C. & HALLAL, P. C. 2007. Gender differences in leisure-time physical activity. *International journal of public health,* 52**,** 8.

BAILEY, D. P. & LOCKE, C. D. 2015. Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *Journal of science and medicine in sport,* 18**,** 294-298.

BARTHOLDY, C., CHRISTENSEN, R., KRISTENSEN, L. E., GUDBERGSEN, H., BLIDDAL, H., OVERGAARD, A., RASMUSSEN, M. U. & HENRIKSEN, M. 2020. Association Between Weight Loss and Spontaneous Changes in Physical Inactivity in Overweight/Obese Individuals With Knee Osteoarthritis: An Eight-Week Prospective Cohort Study. *Arthritis Care & Research,* 72**,** 397-404.

BECK, A. T., WARD, C. H., MENDELSON, M., MOCK, J. & ERBAUGH, J. 1961. An Inventory for Measuring Depression. *Archives of General Psychiatry,* 4**,** 561-571.

BEDOGNI, G., BELLENTANI, S., MIGLIOLI, L., MASUTTI, F., PASSALACQUA, M., CASTIGLIONE, A. & TIRIBELLI, C. 2006. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC gastroenterology,* 6**,** 33.

BELLENTANI, S. 2017. The epidemiology of non-alcoholic fatty liver disease. *Liver International,* 37**,** 81-84.

BELLENTANI, S., DALLE GRAVE, R., SUPPINI, A., MARCHESINI, G. & NETWORK, F. L. I. 2008. Behavior therapy for nonalcoholic fatty liver disease: the need for a multidisciplinary approach. *Hepatology,* 47**,** 746-754.

BELLENTANI, S., SCAGLIONI, F., MARINO, M. & BEDOGNI, G. 2010. Epidemiology of Non-Alcoholic Fatty Liver Disease. *Digestive Diseases,* 28**,** 155-161.

BEN-GAL, T., PINCHAS, A., ZAFRIR, N., SAHAR, G., BERMAN, M. & ARAVOT, D. 2000. Long-term physical training in cardiac transplant candidates: is it feasible? *Transplantation Proceedings,* 32**,** 740-742.

BENHAMOU, P. & PENFORNIS, A. 2002. *Natural history, prognosis, and management of transplantation-induced diabetes mellitus*.

BENSON-DAVIES, S., DAVIES, M. L. & KATTELMANN, K. 2013. Understanding eating and exercise behaviors in post Roux-en-Y gastric bypass patients: a quantitative and qualitative study. *Bariatric surgical practice and patient care,* 8**,** 61-68.

BERGMEYER, H., HORDER, M. & REJ, R. 1986. International Federation Of Clinical Chemistry (Ifcc) 1), 2. *J. Clin. Chem. Clin. Biochem,* 24**,** 481-495.

BERNAL, W., MARTIN-MATEOS, R., LIPCSEY, M., TALLIS, C., WOODSFORD, K., MCPHAIL MARK, J., WILLARS, C., AUZINGER, G., SIZER, E., HENEGHAN, M., COTTAM, S., HEATON, N. & WENDON, J. 2013. Aerobic capacity during cardiopulmonary exercise testing and survival with and without liver transplantation for patients with chronic liver disease. *Liver Transplantation,* 20**,** 54-62.

BERZIGOTTI, A., ALBILLOS, A., VILLANUEVA, C., GENESCÁ, J., ARDEVOL, A., AUGUSTÍN, S., CALLEJA, J. L., BAÑARES, R., GARCÍA-PAGÁN, J. C., MESONERO, F., BOSCH, J. & ON BEHALF OF THE CIBEREHD SPORTDIET COLLABORATIVE, G. 2017. Effects of an intensive lifestyle intervention program on portal hypertension in patients with cirrhosis and obesity: The SportDiet study. *Hepatology,* 65**,** 1293-1305.

BHALA, N., AITHAL, G. & FERGUSON, J. 2013. How to tackle rising rates of liver disease in the UK. *BMJ : British Medical Journal,* 346.

BIOLATO, M., MANCA, F., MARRONE, G., CEFALO, C., RACCO, S., MIGGIANO, G. A., VALENZA, V., GASBARRINI, A., MIELE, L. & GRIECO, A. 2019. Intestinal permeability after Mediterranean diet and low-fat diet in non-alcoholic fatty liver disease. *World journal of gastroenterology,* 25**,** 509.

BIRKENFELD ANDREAS, L. & SHULMAN GERALD, I. 2013. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 Diabetes. *Hepatology,* 59**,** 713-723.

BLANK, V., PETROFF, D., BEER, S., BÖHLIG, A., HENI, M., BERG, T., BAUSBACK, Y., DIETRICH, A., TÖNJES, A. & HOLLENBACH, M. 2020. Current NAFLD guidelines for risk stratification in diabetic patients have poor diagnostic discrimination. *Scientific reports,* 10**,** 1-11.

BLOOMGARDEN, Z. T. 2006. Measures of insulin sensitivity. *Clinics in laboratory medicine,* 26**,** 611-633.

BONORA, E., TARGHER, G., ALBERICHE, M., BONADONNA, R. C., SAGGIANI, F., ZENERE, M. B., MONAUNI, T. & MUGGEO, M. 2000. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes care,* 23**,** 57-63.

BOSY-WESTPHAL, A., JENSEN, B., BRAUN, W., POURHASSAN, M., GALLAGHER, D. & MÜLLER, M. J. 2017. Quantification of whole-body and segmental skeletal muscle mass using phase-sensitive 8-electrode medical bioelectrical impedance devices. *European Journal of Clinical Nutrition,* 71**,** 1061-1067.

BOURSIER, J., VERGNIOL, J., GUILLET, A., HIRIART, J.-B., LANNES, A., LE BAIL, B., MICHALAK, S., CHERMAK, F., BERTRAIS, S., FOUCHER, J., OBERTI, F., CHARBONNIER, M., FOUCHARD-HUBERT, I., ROUSSELET, M.-C., CALÈS, P. & DE LÉDINGHEN, V. 2016. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. *Journal of Hepatology,* 65**,** 570-578.

BRAINARD, J., COOKE, R., LANE, K. & SALTER, C. 2020. Physical activity and retirement: original analysis of responses to the English Adult Active Lives Survey. *International Journal of Public Health,* 65**,** 871-880.

BRAUN, V. & CLARKE, V. 2006. Using thematic analysis in psychology. *Qualitative Research in Psychology,* 3**,** 77-101.

BROWNE, R. H. 1995. On the use of a pilot sample for sample size determination. *Statistics in Medicine,* 14**,** 1933-1940.

BROWNING, J. D., SZCZEPANIAK, L. S., DOBBINS, R., NUREMBERG, P., HORTON, J. D., COHEN, J. C., GRUNDY, S. M. & HOBBS, H. H. 2004. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology,* 40**,** 1387-95.

BUFFENSTEIN, R., KARKLIN, A. & DRIVER, H. S. 2000. Beneficial physiological and performance responses to a month of restricted energy intake in healthy overweight women. *Physiology & Behavior,* 68**,** 439-444.

BURRA, P., BURROUGHS, A., GRAZIADEI, I., PIRENNE, J., VALDECASAS, J. C., MUIESAN, P., SAMUEL, D. & FORNS, X. 2016. EASL clinical practice guidelines: liver transplantation. *Journal of hepatology,* 64**,** 433-485.

BUZZETTI, E., PINZANI, M. & TSOCHATZIS, E. A. 2016. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism,* 65**,** 1038-1048.

BYRNE, S., COOPER, Z. & FAIRBURN, C. 2003. Weight maintenance and relapse in obesity: a qualitative study. *International journal of obesity,* 27**,** 955-962.

CAMPILLO, B., FOUET, P., BONNET, J. C. & ATLAN, G. 1990. Submaximal oxygen consumption in liver cirrhosis: Evidence of severe functional aerobic impairment. *Journal of Hepatology,* 10**,** 163-167.

CAREY, M., SMALL, H., YOONG, S. L., BOYES, A., BISQUERA, A. & SANSON-FISHER, R. 2014. Prevalence of comorbid depression and obesity in general practice: a cross-sectional survey. *British Journal of General Practice,* 64**,** e122.

CASSIDY, S., FULLER, H., CHAU, J., CATT, M., BAUMAN, A. & TRENELL, M. I. 2018. Accelerometer-derived physical activity in those with cardio-metabolic disease compared to healthy adults: a UK Biobank study of 52,556 participants. *Acta diabetologica,* 55**,** 975-979.

CASTÉRA, L., FOUCHER, J., BERNARD, P. H., CARVALHO, F., ALLAIX, D., MERROUCHE, W., COUZIGOU, P. & DE LÉDINGHEN, V. 2010. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology,* 51**,** 828-835.

CENTIS, E., MOSCATIELLO, S., BUGIANESI, E., BELLENTANI, S., FRACANZANI, A. L., CALUGI, S., PETTA, S., DALLE GRAVE, R. & MARCHESINI, G. 2013. Stage of change and motivation to healthier lifestyle in non-alcoholic fatty liver disease. *Journal of Hepatology,* 58**,** 771-777.

CHALASANI, N., YOUNOSSI, Z., LAVINE, J. E., CHARLTON, M., CUSI, K., RINELLA, M., HARRISON, S. A., BRUNT, E. M. & SANYAL, A. J. 2018. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology,* 67**,** 328-357.

CHALASANI, N., YOUNOSSI, Z., LAVINE, J. E., DIEHL, A. M., BRUNT, E. M., CUSI, K., CHARLTON, M. & SANYAL, A. J. 2012. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology,* 142**,** 1592-609.

CHARMAN, S. J., VAN HEES, V. T., QUINN, L., DUNFORD, J. R., BAWAMIA, B., VEERASAMY, M., TRENELL, M. I., JAKOVLJEVIC, D. G. & KUNADIAN, V. 2016. The effect of percutaneous coronary intervention on habitual physical activity in older patients. *BMC cardiovascular disorders,* 16**,** 248.

CHEN, S. & YAN, E. 2006. Very-Low-Calorie Diet Revisited. *Obesity Management,* 2**,** 31-32.

CHEYETTE, B. C. 2010. *Carbs & Cals: Carb & Calorie Counter.,* Great Britain, Chello Publishing Ltd.

CHEYETTE, C. B. 2017. *Carbs & Cals: Very Low Calorie Recipes and Meal Plans.,* Great Britain, Chello Publishing Ltd.

CHIDA, Y. & STEPTOE, A. 2008. Positive Psychological Well-Being and Mortality: A Quantitative Review of Prospective Observational Studies. *Psychosomatic Medicine,* 70**,** 741-756.

CHIRINOS, J. A., GURUBHAGAVATULA, I., TEFF, K., RADER, D. J., WADDEN, T. A., TOWNSEND, R., FOSTER, G. D., MAISLIN, G., SAIF, H. & BRODERICK, P. 2014. CPAP, weight loss, or both for obstructive sleep apnea. *New England Journal of Medicine,* 370**,** 2265-2275.

CHOLONGITAS, E., MARELLI, L., SHUSANG, V., SENZOLO, M., ROLLES, K., PATCH, D. & BURROUGHS, A. K. 2006. A systematic review of the performance of the model for end-stage liver disease (MELD) in the setting of liver transplantation. *Liver Transplantation,* 12**,** 1049-1061.

CHOMISTEK, A. K., YUAN, C., MATTHEWS, C. E., TROIANO, R. P., BOWLES, H. R., ROOD, J., BARNETT, J. B., WILLETT, W. C., RIMM, E. B. & BASSETT JR, D. R. 2017. Physical activity assessment with the ActiGraph GT3X and doubly labeled water. *Medicine and science in sports and exercise,* 49**,** 1935.

CHONG, C. Y. L., ORR, D., PLANK, L. D., VATANEN, T., O'SULLIVAN, J. M. & MURPHY, R. 2020. Randomised Double-Blind Placebo-Controlled Trial of Inulin with Metronidazole in Non-Alcoholic Fatty Liver Disease (NAFLD). *Nutrients,* 12**,** 937.

CLARK, B. C., CLARK, L. A. & LAW, T. D. 2016. Resistance exercise to prevent and manage sarcopenia and dynapenia. *Annual Review of Gerontology and Geriatrics,* 36**,** 205-228.

COLLES, S. L., DIXON, J. B., MARKS, P., STRAUSS, B. J. & O'BRIEN, P. E. 2006. Preoperative weight loss with a very-low-energy diet: quantitation of changes in liver and abdominal fat by serial imaging–. *The American journal of clinical nutrition,* 84**,** 304-311.

COLLIER, J. 2007. Bone disorders in chronic liver disease. *Hepatology,* 46**,** 1271-1278.

COLLINS, C. 2003. Survey of dietetic management of overweight and obesity and comparison with best practice criteria. *Nutrition and Dietetics,* 60**,** 177-184.

COREY KATHLEEN, E. & VUPPALANCHI, R. 2012. Assessment and management of comorbidities (including cardiovascular disease) in patients with nonalcoholic fatty liver disease. *Clinical Liver Disease,* 1**,** 114-116.

CRAFT, L. L. & LANDERS, D. M. 1998. The effect of exercise on clinical depression and depression resulting from mental illness: A meta-analysis. *Journal of Sport and Exercise Psychology,* 20**,** 339-357.

CURRAN, K., DRUST, B., MURPHY, R., PRINGLE, A. & RICHARDSON, D. 2016. The challenge and impact of engaging hard-to-reach populations in regular physical activity and health behaviours: an examination of an English Premier League 'Football in the Community'men's health programme. *Public Health,* 135**,** 14-22.

DAVIS, P. D., DENNIS, J. L. & RAILTON, R. 2005. Evaluation of the A&D UA-767 and Welch Allyn Spot Vital Signs noninvasive blood pressure monitors using a blood pressure simulator. *Journal of Human Hypertension,* 19**,** 197-203.

DAY, C. P. 2002. Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? *Gut,* 50**,** 585.

DAY, C. P. 2006. Non-alcoholic fatty liver disease: current concepts and management strategies. *Clinical Medicine,* 6**,** 19-25.

DAY, C. P. 2011. Non-alcoholic fatty liver disease: a massive problem. *Clin Med (Lond),* 11**,** 176-8.

DAY, C. P. & JAMES, O. F. W. Steatohepatitis: A tale of two hits. *Gastroenterology,* 114**,** 842-845.

DE LUCA, L., WESTBROOK, R. & TSOCHATZIS, E. A. 2015. Metabolic and cardiovascular complications in the liver transplant recipient. *Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology,* 28**,** 183.

DE MOOR, M. H. M., BEEM, A. L., STUBBE, J. H., BOOMSMA, D. I. & DE GEUS, E. J. C. 2006. Regular exercise, anxiety, depression and personality: A population-based study. *Preventive Medicine,* 42**,** 273-279.

DEBETTE-GRATIEN, M., TABOURET, T., ANTONINI, M. T., DALMAY, F., CARRIER, P., LEGROS, R., JACQUES, J., VINCENT, F., SAUTEREAU, D., SAMUEL, D. & LOUSTAUD-RATTI, V. 2015. Personalized adapted physical activity before liver transplantation: acceptability and results. *Transplantation,* 99**,** 145-50.

DHARANCY, S., LEMYZE, M., BOLESLAWSKI, E., NEVIERE, R., DECLERCK, N., CANVA, V., WALLAERT, B., MATHURIN, P. & PRUVOT, F. R. 2008. Impact of impaired aerobic capacity on liver transplant candidates. *Transplantation,* 86**,** 1077-1083.

DICK ANDRÉ, A. S., SPITZER AUSTIN, L., SEIFERT CATHERINE, F., DECKERT, A., CARITHERS ROBERT, L., REYES JORGE, D. & PERKINS JAMES, D. 2009. Liver transplantation at the extremes of the body mass index. *Liver Transplantation,* 15**,** 968-977.

DIMARTINI, A., CRUZ JR, R. J., DEW, M. A., MYASKOVSKY, L., GOODPASTER, B., FOX, K., KIM, K. H. & FONTES, P. 2013. Muscle mass predicts outcomes following liver transplantation. *Liver Transplantation,* 19**,** 1172-1180.

DISEASE, N. I. O. D. A. D. A. K. 2012. *National Institute of Diabetes and Digestive and Kidney Disease. Very low-calorie diets* [Online]. Available: http://www.niddk.nih.gov/health-information/health-topics/weight-control/very-low-calorie-diets/Documents/verylowcaldietsbw.pdf [Accessed 11/02/2020 2020].

DIXON, J. B., SCHACHTER, L. M., O'BRIEN, P. E., JONES, K., GRIMA, M., LAMBERT, G., BROWN, W., BAILEY, M. & NAUGHTON, M. T. 2012. Surgical vs conventional therapy for weight loss treatment of obstructive sleep apnea: a randomized controlled trial. *Jama,* 308**,** 1142-1149.

DLTR 2000. Index of Multiple Deprivation. DLTR London.

DOHERTY, A., JACKSON, D., HAMMERLA, N., PLÖTZ, T., OLIVIER, P., GRANAT, M. H., WHITE, T., VAN HEES, V. T., TRENELL, M. I. & OWEN, C. G. 2017. Large scale population

assessment of physical activity using wrist worn accelerometers: The UK Biobank Study. *PloS one,* 12**,** e0169649.

DUARTE-ROJO, A., BLOOMER, P. M., ROGERS, R. J., HASSAN, M. A., DUNN, M. A., TEVAR, A. D., VIVIS, S. L., BATALLER, R., HUGHES, C. B., FERRANDO, A. A., JAKICIC, J. M. & RAY KIM, W. 2020. Introducing EL-FIT (Exercise & Liver FITness), a smartphone app to prehabilitate and monitor liver transplant candidates. *Liver Transplantation,* n/a.

DUARTE-ROJO, A., RUIZ-MARGÁIN, A., MONTAÑO-LOZA, A. J., MACÍAS-RODRÍGUEZ, R. U., FERRANDO, A. & KIM, W. R. 2018. Exercise and physical activity for patients with end-stage liver disease: Improving functional status and sarcopenia while on the transplant waiting list. *Liver Transplantation,* 24**,** 122-139.

DUARTE-ROJO, A., RUIZ-MARGÁIN, A., MONTAÑO-LOZA, A. J., MACÍAS-RODRÍGUEZ, R. U., FERRANDO, A. & KIM, W. R. 2018. Exercise and physical activity for patients with end-stage liver disease: improving functional status and sarcopenia while on the transplant waiting list. *Liver Transplantation,* 24**,** 122-139.

DUNN, M. A., ROGAL, S. S., DUARTE-ROJO, A. & LAI, J. C. 2020. Physical Function, Physical Activity, and Quality of Life After Liver Transplantation. *Liver Transplantation,* 26**,** 702-708.

DYSON, J. K., ANSTEE, Q. M. & MCPHERSON, S. 2014. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. *Frontline Gastroenterology,* 5**,** 211.

ECKARD, C., COLE, R., LOCKWOOD, J., TORRES, D. M., WILLIAMS, C. D., SHAW, J. C. & HARRISON, S. A. 2013. Prospective histopathologic evaluation of lifestyle modification in nonalcoholic fatty liver disease: a randomized trial. *Therap Adv Gastroenterol,* 6**,** 249-59.

EIN, N., ARMSTRONG, B. & VICKERS, K. 2019. The effect of a very low calorie diet on subjective depressive symptoms and anxiety: meta-analysis and systematic review. *International Journal of Obesity,* 43**,** 1444-1455.

EKELUND, U., TARP, J., STEENE-JOHANNESSEN, J., HANSEN, B. H., JEFFERIS, B., FAGERLAND, M. W., WHINCUP, P., DIAZ, K. M., HOOKER, S. P. & CHERNOFSKY, A. 2019. Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: systematic review and harmonised meta-analysis. *bmj,* 366**,** l4570.

EKSTEDT, M., FRANZÉN, L. E., MATHIESEN, U. L., THORELIUS, L., HOLMQVIST, M., BODEMAR, G. & KECHAGIAS, S. 2006. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology,* 44**,** 865-873.

ENGLAND, P. H. 2013. *NHS Health Check implementation review and action plan. Public Health England.* [Online]. Available: https://www.gov.uk/government/publications/nhs-health-check-implementation-review-and-action-plan [Accessed].

ENGLESBE, M. J., PATEL, S. P., HE, K., LYNCH, R. J., SCHAUBEL, D. E., HARBAUGH, C., HOLCOMBE, S. A., WANG, S. C., SEGEV, D. L. & SONNENDAY, C. J. 2010. Sarcopenia and mortality after liver transplantation. *Journal of the American College of Surgeons,* 211**,** 271-278.

ENGSTRÖM, M. & FORSBERG, A. 2011. Wishing for deburdening through a sustainable control after bariatric surgery. *International Journal of Qualitative Studies on Health and Well-being,* 6**,** 5901.

ESLAMPARAST, T., MONTANO-LOZA, A. J., RAMAN, M. & TANDON, P. 2018. Sarcopenic obesity in cirrhosis—The confluence of 2 prognostic titans. *Liver International,* 38**,** 1706-1717.

ESTES, C., RAZAVI, H., LOOMBA, R., YOUNOSSI, Z. & SANYAL, A. J. 2018. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology,* 67**,** 123-133.

FELDSTEIN, A. E., WERNEBURG, N. W., CANBAY, A., GUICCIARDI, M. E., BRONK, S. F., RYDZEWSKI, R., BURGART, L. J. & GORES, G. J. 2004. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-α expression via a lysosomal pathway. *Hepatology,* 40**,** 185-194.

FERREIRA, R. & MURRAY, J. 1983. Spielberger's State-Trait Anxiety Inventory: Measuring anxiety with and without an audience during performance on a stabilometer. *Percept Mot Skills,* 57**,** 15-8.

FLOREZ, H., LUO, J., CASTILLO-FLOREZ, S., MITSI, G., HANNA, J., TAMARIZ, L., PALACIO, A., NAGENDRAN, S. & HAGAN, M. 2010. Impact of Metformin-Induced Gastrointestinal Symptoms on Quality of Life and Adherence in Patients with Type 2 Diabetes. *Postgraduate Medicine,* 122**,** 112-120.

FOGELHOLM, M., KUKKONEN-HARJULA, K. & OJA, P. 1999. Eating control and physical activity as determinants of short-term weight maintenance after a very-low-calorie diet among obese women. *International journal of obesity,* 23**,** 203-210.

FOSTER, G. D., BORRADAILE, K. E., SANDERS, M. H., MILLMAN, R., ZAMMIT, G., NEWMAN, A. B., WADDEN, T. A., KELLEY, D., WING, R. R. & PI-SUNYER, F. X. 2009. A randomized study on the effect of weight loss on obstructive sleep apnea among obese patients with type 2 diabetes: the Sleep AHEAD study. *Archives of internal medicine,* 169**,** 1619-1626.

FRACANZANI, A. L., VALENTI, L., BUGIANESI, E., ANDREOLETTI, M., COLLI, A., VANNI, E., BERTELLI, C., FATTA, E., BIGNAMINI, D. & MARCHESINI, G. 2008. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology,* 48**,** 792-798.

FRANZ, M. J., VANWORMER, J. J., CRAIN, A. L., BOUCHER, J. L., HISTON, T., CAPLAN, W., BOWMAN, J. D. & PRONK, N. P. 2007. Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. *Journal of the American Dietetic association,* 107**,** 1755-1767.

FRIEDMAN, S., SANYAL, A., GOODMAN, Z., LEFEBVRE, E., GOTTWALD, M., FISCHER, L. & RATZIU, V. 2016. Efficacy and safety study of cenicriviroc for the treatment of non-alcoholic steatohepatitis in adult subjects with liver fibrosis: CENTAUR Phase 2b study design. *Contemp Clin Trials,* 47**,** 356-65.

FRIS, R. J. 2004. Preoperative low energy diet diminishes liver size. *Obesity surgery,* 14**,** 1165-1170.

FRITH, J., DAY, C. P., ROBINSON, L., ELLIOTT, C., JONES, D. E. & NEWTON, J. L. 2010. Potential strategies to improve uptake of exercise interventions in non-alcoholic fatty liver disease. *Journal of hepatology,* 52**,** 112-116.

FUNNELL, M. M. & ANDERSON, R. M. 2004. Empowerment and self-management of diabetes. *Clinical diabetes,* 22**,** 123-127.

GASTALDELLI, A., CUSI, K., PETTITI, M., HARDIES, J., MIYAZAKI, Y., BERRIA, R., BUZZIGOLI, E., SIRONI, A. M., CERSOSIMO, E., FERRANNINI, E. & DEFRONZO, R. A. 2007. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology,* 133**,** 496-506.

GEORGE, J. & LIDDLE, C. 2008. Nonalcoholic Fatty Liver Disease: Pathogenesis and Potential for Nuclear Receptors as Therapeutic Targets. *Molecular Pharmaceutics,* 5**,** 49-59.

GEPNER, Y., SHELEF, I., KOMY, O., COHEN, N., SCHWARZFUCHS, D., BRIL, N., REIN, M., SERFATY, D., KENIGSBUCH, S., ZELICHA, H., YASKOLKA MEIR, A., TENE, L., BILITZKY, A., TSABAN, G., CHASSIDIM, Y., SARUSY, B., CEGLAREK, U., THIERY, J., STUMVOLL, M., BLÜHER, M., STAMPFER, M. J., RUDICH, A. & SHAI, I. 2019. The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content. *Journal of Hepatology,* 71**,** 379-388.

GERBER, L., OTGONSUREN, M., MISHRA, A., ESCHEIK, C., BIRERDINC, A., STEPANOVA, M. & YOUNOSSI, Z. 2012. Non-alcoholic fatty liver disease (NAFLD) is associated with low level of physical activity: a population-based study. *Alimentary pharmacology & therapeutics,* 36**,** 772-781.

GHAEMI, A., TALEBAN, F. A., HEKMATDOOST, A., RAFIEI, A., HOSSEINI, V., AMIRI, Z., HOMAYOUNFAR, R. & FAKHERI, H. 2013. How Much Weight Loss is Effective on Nonalcoholic Fatty Liver Disease? *Hepatitis Monthly,* 13**,** e15227.

GISBERT, C., PRIETO, M., BERENGUER, M., BRETÓ, M., CARRASCO, D., DE JUAN, M., MIR, J. & BERENGUER, J. 1997. Hyperlipidemia in liver transplant recipients: prevalence and risk factors. *Liver Transplantation and Surgery,* 3**,** 416-422.

GLASS, L. M., DICKSON, R. C., ANDERSON, J. C., SURIAWINATA, A. A., PUTRA, J., BERK, B. S. & TOOR, A. 2015. Total body weight loss of >/= 10 % is associated with improved hepatic fibrosis in patients with nonalcoholic steatohepatitis. *Dig Dis Sci,* 60**,** 1024-30.

GLEN, J., FLOROS, L., DAY, C. & PRYKE, R. 2016. Non-alcoholic fatty liver disease (NAFLD): summary of NICE guidance. *Bmj,* 354**,** i4428.

GOLABI, P., OTGONSUREN, M., CABLE, R., FELIX, S., KOENIG, A., SAYINER, M. & YOUNOSSI, Z. M. 2016. Non-alcoholic fatty liver disease (NAFLD) is associated with impairment of health related quality of life (HRQOL). *Health and quality of life outcomes,* 14**,** 18.

GREAVES, C., POLTAWSKI, L., GARSIDE, R. & BRISCOE, S. 2017. Understanding the challenge of weight loss maintenance: a systematic review and synthesis of qualitative research on weight loss maintenance. *Health Psychology Review,* 11**,** 145-163.

GREEN, A., LARKIN, M. & SULLIVAN, V. 2009. Oh stuff it! The experience and explanation of diet failure: an exploration using interpretative phenomenological analysis. *Journal of Health Psychology,* 14**,** 997-1008.

GREGORY, A. M., BUYSSE, D. J., WILLIS, T. A., RIJSDIJK, F. V., MAUGHAN, B., ROWE, R., CARTWRIGHT, S., BARCLAY, N. L. & ELEY, T. C. 2011. Associations between sleep quality and anxiety and depression symptoms in a sample of young adult twins and siblings. *Journal of Psychosomatic Research,* 71**,** 250-255.

GRUNDY, A., COTTERCHIO, M., KIRSH, V. A. & KREIGER, N. 2014. Associations between Anxiety, Depression, Antidepressant Medication, Obesity and Weight Gain among Canadian Women. *PLOS ONE,* 9**,** e99780.

HAGSTRÖM, H., NASR, P., EKSTEDT, M., HAMMAR, U., STÅL, P., HULTCRANTZ, R. & KECHAGIAS, S. 2017. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. *Journal of Hepatology,* 67**,** 1265-1273.

HAIGH, L., BREMNER, S., HOUGHTON, D., HENDERSON, E., AVERY, L., HARDY, T., HALLSWORTH, K., MCPHERSON, S. & ANSTEE, Q. M. 2019. Barriers and facilitators to Mediterranean diet adoption by patients with nonalcoholic fatty liver disease in Northern Europe. *Clinical Gastroenterology and Hepatology,* 17**,** 1364-1371. e3.

HALLSWORTH, K., DOMBROWSKI, S. U., MCPHERSON, S., ANSTEE, Q. M. & AVERY, L. 2019. Using the theoretical domains framework to identify barriers and enabling factors to implementation of guidance for the diagnosis and management of nonalcoholic fatty liver disease: a qualitative study. *Translational Behavioral Medicine*.

HALLSWORTH, K., FATTAKHOVA, G., HOLLINGSWORTH, K. G., THOMA, C., MOORE, S., TAYLOR, R., DAY, C. P. & TRENELL, M. I. 2011. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut,* 60**,** 1278.

HALLSWORTH, K., THOMA, C., MOORE, S., PLOETZ, T., ANSTEE, Q. M., TAYLOR, R., DAY, C. P. & TRENELL, M. I. 2015. Non-alcoholic fatty liver disease is associated with higher levels of objectively measured sedentary behaviour and lower levels of physical activity than matched healthy controls. *Frontline gastroenterology,* 6**,** 44-51.

HANSEN, B. H., HOLME, I., ANDERSSEN, S. A. & KOLLE, E. 2013. Patterns of objectively measured physical activity in normal weight, overweight, and obese individuals (20–85 years): a cross-sectional study. *PLoS One,* 8**,** e53044.

HART, T. L., SWARTZ, A. M., CASHIN, S. E. & STRATH, S. J. 2011. How many days of monitoring predict physical activity and sedentary behaviour in older adults? *International Journal of Behavioral Nutrition and Physical Activity,* 8**,** 62.

HAUFE, S., ENGELI, S., KAST, P., BOHNKE, J., UTZ, W., HAAS, V., HERMSDORF, M., MAHLER, A., WIESNER, S., BIRKENFELD, A. L., SELL, H., OTTO, C., MEHLING, H., LUFT, F. C., ECKEL, J., SCHULZ-MENGER, J., BOSCHMANN, M. & JORDAN, J. 2011a. Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. *Hepatology,* 53**,** 1504-14.

HAUFE, S., ENGELI, S., KAST, P., BÖHNKE, J., UTZ, W., HAAS, V., HERMSDORF, M., MÄHLER, A., WIESNER, S., BIRKENFELD, A. L., SELL, H., OTTO, C., MEHLING, H., LUFT, F. C., ECKEL, J., SCHULZ-MENGER, J., BOSCHMANN, M. & JORDAN, J. 2011b. Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. *Hepatology,* 53**,** 1504-1514.

HAUGEN, C. E., MCADAMS-DEMARCO, M., HOLSCHER, C. M., YING, H., GURAKAR, A. O., GARONZIK-WANG, J., CAMERON, A. M., SEGEV, D. L. & LAI, J. C. 2020. Multicenter Study of Age, Frailty, and Waitlist Mortality Among Liver Transplant Candidates. *Annals of Surgery,* 271.

HAYES, K., LEET, A. S., BRADLEY, S. J. & HOLLAND, A. E. 2012. Effects of exercise training on exercise capacity and quality of life in patients with a left ventricular assist device: A preliminary randomized controlled trial. *The Journal of Heart and Lung Transplantation,* 31**,** 729-734.

HEALTH, N. I. O. 1990. Recommendations for improving cholesterol measurement: a report from the Laboratory Standardization Panel of the National Cholesterol Education Program. *Bethesda, MD: National Institutes of Health*.

HERRIOT, A., THOMAS, D., HART, K., WARREN, J. & TRUBY, H. 2008. A qualitative investigation of individuals' experiences and expectations before and after completing a trial of commercial weight loss programmes. *Journal of Human Nutrition and Dietetics,* 21**,** 72-80.

HILDEBRAND, M., VAN, V. H., HANSEN, B. H. & EKELUND, U. 2014. Age group comparability of raw accelerometer output from wrist-and hip-worn monitors. *Medicine and science in sports and exercise,* 46**,** 1816-1824.

HIPPISLEY-COX, J., COUPLAND, C., VINOGRADOVA, Y., ROBSON, J., MINHAS, R., SHEIKH, A. & BRINDLE, P. 2008. Predicting cardiovascular risk in England and Wales: prospective derivation and validation of QRISK2. *Bmj,* 336**,** 1475-1482.

HOWE, C. A., STAUDENMAYER, J. W. & FREEDSON, P. S. 2009. Accelerometer prediction of energy expenditure: vector magnitude versus vertical axis. *Med Sci Sports Exerc,* 41**,** 2199-206.

HUANG, P. L. 2009. A comprehensive definition for metabolic syndrome. *Disease models & mechanisms,* 2**,** 231-237.

HUBER, Y., BOYLE, M., HALLSWORTH, K., TINIAKOS, D., STRAUB, B. K., LABENZ, C., RUCKES, C., GALLE, P. R., ROMERO-GÓMEZ, M., ANSTEE, Q. M. & SCHATTENBERG, J. M. 2019. Health-related Quality of Life in Nonalcoholic Fatty Liver Disease Associates With Hepatic Inflammation. *Clinical Gastroenterology and Hepatology,* 17**,** 2085-2092.e1.

IMAIZUMI, H., TAKAHASHI, A., TANJI, N., ABE, K., SATO, Y., ANZAI, Y., WATANABE, H. & OHIRA, H. 2015. The association between sleep duration and non-alcoholic fatty liver disease among Japanese men and women. *Obesity facts,* 8**,** 234-242.

JACKNESS, C., KARMALLY, W., FEBRES, G., CONWELL, I. M., AHMED, L., BESSLER, M., MCMAHON, D. J. & KORNER, J. 2013. Very Low–Calorie diet mimics the early beneficial effect of Roux-en-Y gastric bypass on insulin sensitivity and β-cell function in type 2 diabetic patients. *Diabetes,* 62**,** 3027-3032.

JALLINOJA, P., PAJARI, P. & ABSETZ, P. 2008. Repertoires of lifestyle change and self-responsibility among participants in an intervention to prevent type 2 diabetes. *Scandinavian journal of caring sciences,* 22**,** 455-462.

JANUS, C., JENSEN, S., LUNDGREN, J. R., JUHL, C. R., OLSEN, L. M., STALLKNECHT, B., HOLST, J. J., MADSBAD, S. & TOREKOV, S. S. 2020. 1974-P: Diet-Induced Weight Loss Improves Sleep Quality and These Improvements Are Sustained with One Year of Weight Maintenance with Exercise: The S-LITE Randomized Trial. Am Diabetes Assoc.

JEBB, S. A., ASTBURY, N. M., TEARNE, S., NICKLESS, A. & AVEYARD, P. 2017. Doctor Referral of Overweight People to a Low-Energy Treatment (DROPLET) in primary care using

total diet replacement products: a protocol for a randomised controlled trial. *BMJ Open,* 7**,** e016709.

JENSEN, M. D. & RYAN, D. H. 2014. New obesity guidelines: promise and potential. *Jama,* 311**,** 23-24.

JOHANSSON, K., NEOVIUS, M. & HEMMINGSSON, E. 2014. Effects of anti-obesity drugs, diet, and exercise on weight-loss maintenance after a very-low-calorie diet or low-calorie diet: a systematic review and meta-analysis of randomized controlled trials. *The American journal of clinical nutrition,* 99**,** 14-23.

JOHNSON, N. A., SACHINWALLA, T., WALTON, D. W., SMITH, K., ARMSTRONG, A., THOMPSON, M. W. & GEORGE, J. 2009. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology,* 50**,** 1105-1112.

JOHNSON, T. M., OVERGARD, E. B., COHEN, A. E. & DIBAISE, J. K. 2013. Nutrition Assessment and Management in Advanced Liver Disease. *Nutrition in Clinical Practice,* 28**,** 15-29.

KAIDO, T., TAMAI, Y., HAMAGUCHI, Y., OKUMURA, S., KOBAYASHI, A., SHIRAI, H., YAGI, S., KAMO, N., HAMMAD, A., INAGAKI, N. & UEMOTO, S. 2017. Effects of pretransplant sarcopenia and sequential changes in sarcopenic parameters after living donor liver transplantation. *Nutrition,* 33**,** 195-198.

KANSANEN, M., VANNINEN, E., TUUNAINEN, A., PESONEN, P., TUONONEN, V., HARTIKAINEN, J., MUSSALO, H. & UUSITUPA, M. 1998. The effect of a very low-calorie diet-induced weight loss on the severity of obstructive sleep apnoea and autonomic nervous function in obese patients with obstructive sleep apnoea syndrome. *Clinical Physiology,* 18**,** 377-385.

KANTARTZIS, K., PETER, A., MACHICAO, F., MACHANN, J., WAGNER, S., KÖNIGSRAINER, I., KÖNIGSRAINER, A., SCHICK, F., FRITSCHE, A. & HÄRING, H.-U. 2009. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes,* 58**,** 2616-2623.

KAPLAN, L. A., PESCE, A. J. & KAZMIERCZAK, S. C. 2003. *Clinical chemistry: theory, analysis, correlation*, Mosby Incorporated.

KÁRASON, H. & VAN DER HILST, R. D. 2000. Constraints on mantle convection from seismic tomography. *Geophysical monograph,* 121**,** 277-288.

KATSAGONI, C. N., PAPATHEODORIDIS, G. V., IOANNIDOU, P., DEUTSCH, M., ALEXOPOULOU, A., PAPADOPOULOS, N., PAPAGEORGIOU, M.-V., FRAGOPOULOU, E. & KONTOGIANNI, M. D. 2018. Improvements in clinical characteristics of patients with non-alcoholic fatty liver disease, after an intervention based on the Mediterranean lifestyle: a randomised controlled clinical trial. *British Journal of Nutrition,* 120**,** 164-175.

KEMMER, N., ALSINA, A. & NEFF, G. W. 2011. Social Determinants of Orthotopic Liver Transplantation Candidacy: Role of Patient-Related Factors. *Transplantation Proceedings,* 43**,** 3769-3772.

KENT, S., AVEYARD, P., ASTBURY, N., MIHAYLOVA, B. & JEBB, S. A. 2019. Is Doctor Referral to a Low-Energy Total Diet Replacement Program Cost-Effective for the Routine Treatment of Obesity? *Obesity,* 27**,** 391-398.

KESKIN, M., KURTOGLU, S., KENDIRCI, M., ATABEK, M. E. & YAZICI, C. 2005. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. *Pediatrics,* 115**,** e500-e503.

KIM, M. & KIM, H. 2013. Accuracy of segmental multi-frequency bioelectrical impedance analysis for assessing whole-body and appendicular fat mass and lean soft tissue mass in frail women aged 75 years and older. *European journal of clinical nutrition,* 67**,** 395-400.

KIRK, E., REEDS, D. N., FINCK, B. N., MAYURRANJAN, M. S., PATTERSON, B. W. & KLEIN, S. 2009. Dietary Fat and Carbohydrates Differentially Alter Insulin Sensitivity During Caloric Restriction. *Gastroenterology,* 136**,** 1552-1560.

KISTLER, K. D., BRUNT, E. M., CLARK, J. M., DIEHL, A. M., SALLIS, J. F. & SCHWIMMER, J. B. 2011. Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic fatty liver disease. *Am J Gastroenterol,* 106**,** 460-8; quiz 469.

KLEINER, D. E., BRUNT, E. M., VAN NATTA, M., BEHLING, C., CONTOS, M. J., CUMMINGS, O. W., FERRELL, L. D., LIU, Y.-C., TORBENSON, M. S., UNALP-ARIDA, A., YEH, M., MCCULLOUGH, A. J., SANYAL, A. J. & NONALCOHOLIC STEATOHEPATITIS CLINICAL RESEARCH, N. 2005. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology,* 41**,** 1313-1321.

KNEEMAN, J. M., MISDRAJI, J. & COREY, K. E. 2012. Secondary causes of nonalcoholic fatty liver disease. *Therapeutic Advances in Gastroenterology,* 5**,** 199-207.

KOZLITINA, J., SMAGRIS, E., STENDER, S., NORDESTGAARD, B. G., ZHOU, H. H., TYBJÆRG-HANSEN, A., VOGT, T. F., HOBBS, H. H. & COHEN, J. C. 2014. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nature Genetics,* 46**,** 352-356.

KRAMER, H., PICKHARDT, P. J., KLIEWER, M. A., HERNANDO, D., CHEN, G.-H., ZAGZEBSKI, J. A. & REEDER, S. B. 2017. Accuracy of liver fat quantification with advanced CT, MRI, and ultrasound techniques: prospective comparison with MR spectroscopy. *American journal of Roentgenology,* 208**,** 92-100.

KRASNOFF, J. B., PAINTER, P. L., WALLACE, J. P., BASS, N. M. & MERRIMAN, R. B. 2008. Health-related fitness and physical activity in patients with nonalcoholic fatty liver disease. *Hepatology,* 47**,** 1158-66.

KRESS, J. P., RUBIN, A., POHLMAN, A. S. & HALL, J. B. 2000. Outcomes of Critically Ill Patients Denied Consideration for Liver Transplantation. *American Journal of Respiratory and Critical Care Medicine,* 162**,** 418-423.

KRUGER, C., MCNEELY, M. L., BAILEY, R. J., YAVARI, M., ABRALDES, J. G., CARBONNEAU, M., NEWNHAM, K., DENHEYER, V., MA, M., THOMPSON, R., PATERSON, I., HAYKOWSKY, M. J. & TANDON, P. 2018. Home Exercise Training Improves Exercise Capacity in Cirrhosis Patients: Role of Exercise Adherence. *Scientific Reports,* 8**,** 99.

KWO, P. Y., COHEN, S. M. & LIM, J. K. 2017. ACG clinical guideline: evaluation of abnormal liver chemistries. *American Journal of Gastroenterology,* 112**,** 18-35.

LAI, C. W., MINTO, G., CHALLAND, C. P., HOSIE, K. B., SNEYD, J. R., CREANOR, S. & STRUTHERS, R. A. 2013. Patients' inability to perform a preoperative cardiopulmonary exercise test or demonstrate an anaerobic threshold is associated with inferior outcomes after major colorectal surgery. *BJA: British Journal of Anaesthesia,* 111**,** 607-611.

LAI, J. C., DODGE, J. L., SEN, S., COVINSKY, K. & FENG, S. 2016. Functional decline in patients with cirrhosis awaiting liver transplantation: Results from the functional assessment in liver transplantation (FrAILT) study. *Hepatology,* 63**,** 574-580.

LAI, J. C., FENG, S., TERRAULT, N. A., LIZAOLA, B., HAYSSEN, H. & COVINSKY, K. 2014. Frailty Predicts Waitlist Mortality in Liver Transplant Candidates. *American Journal of Transplantation,* 14**,** 1870-1879.

LAI, J. C., SEGEV, D. L., MCCULLOCH, C. E., COVINSKY, K. E., DODGE, J. L. & FENG, S. 2018. Physical frailty after liver transplantation. *American Journal of Transplantation,* 18**,** 1986-1994.

LAMBERT, E. A., RICE, T., EIKELIS, N., STRAZNICKY, N. E., LAMBERT, G. W., HEAD, G. A., HENSMAN, C., SCHLAICH, M. P. & DIXON, J. B. 2014. Sympathetic activity and markers of cardiovascular risk in nondiabetic severely obese patients: the effect of the initial 10% weight loss. *American journal of hypertension,* 27**,** 1308-1315.

LANCASTER, G. A., DODD, S. & WILLIAMSON, P. R. 2004. Design and analysis of pilot studies: recommendations for good practice. *Journal of Evaluation in Clinical Practice,* 10**,** 307-312.

LASIKIEWICZ, N., MYRISSA, K., HOYLAND, A. & LAWTON, C. L. 2014. Psychological benefits of weight loss following behavioural and/or dietary weight loss interventions. A systematic research review. *Appetite,* 72**,** 123-137.

LAZO, M., HERNAEZ, R., EBERHARDT, M. S., BONEKAMP, S., KAMEL, I., GUALLAR, E., KOTEISH, A., BRANCATI, F. L. & CLARK, J. M. 2013. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988–1994. *American journal of epidemiology,* 178**,** 38-45.

LEAN, M. E., LESLIE, W. S., BARNES, A. C., BROSNAHAN, N., THOM, G., MCCOMBIE, L., PETERS, C., ZHYZHNEUSKAYA, S., AL-MRABEH, A. & HOLLINGSWORTH, K. G. 2018. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *The Lancet,* 391**,** 541-551.

LEAN, M. E. J., LESLIE, W. S., BARNES, A. C., BROSNAHAN, N., THOM, G., MCCOMBIE, L., PETERS, C., ZHYZHNEUSKAYA, S., AL-MRABEH, A., HOLLINGSWORTH, K. G., RODRIGUES, A. M., REHACKOVA, L., ADAMSON, A. J., SNIEHOTTA, F. F., MATHERS, J. C., ROSS, H. M., MCILVENNA, Y., STEFANETTI, R., TRENELL, M., WELSH, P., KEAN, S., FORD, I., MCCONNACHIE, A., SATTAR, N. & TAYLOR, R. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *The Lancet*.

LEAN, M. E. J., LESLIE, W. S., BARNES, A. C., BROSNAHAN, N., THOM, G., MCCOMBIE, L., PETERS, C., ZHYZHNEUSKAYA, S., AL-MRABEH, A., HOLLINGSWORTH, K. G., RODRIGUES, A. M., REHACKOVA, L., ADAMSON, A. J., SNIEHOTTA, F. F., MATHERS, J. C., ROSS, H. M., MCILVENNA, Y., WELSH, P., KEAN, S., FORD, I., MCCONNACHIE, A., MESSOW, C.-M., SATTAR, N. & TAYLOR, R. 2019. Durability of a primary care-led weight-management intervention for remission of type 2 diabetes: 2-year results of the DiRECT open-label, cluster-randomised trial. *The Lancet Diabetes & Endocrinology,* 7**,** 344-355.

LEE, T. A., LI, Z., ZERLIN, A. & HEBER, D. 2010. Effects of dihydrocapsiate on adaptive and diet-induced thermogenesis with a high protein very low calorie diet: a randomized control trial. *Nutrition & metabolism,* 7**,** 78.

LEITE, N. C., SALLES, G. F., ARAUJO, A. L., VILLELA-NOGUEIRA, C. A. & CARDOSO, C. R. 2009. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int,* 29**,** 113-9.

LEITE, N. C., VILLELA-NOGUEIRA, C. A., PANNAIN, V. L., BOTTINO, A. C., REZENDE, G. F., CARDOSO, C. R. & SALLES, G. F. 2011. Histopathological stages of nonalcoholic fatty liver disease in type 2 diabetes: prevalences and correlated factors. *Liver Int,* 31**,** 700-6.

LEONG, D. P., TEO, K. K., RANGARAJAN, S., LOPEZ-JARAMILLO, P., AVEZUM JR, A., ORLANDINI, A., SERON, P., AHMED, S. H., ROSENGREN, A. & KELISHADI, R. 2015. Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. *The Lancet,* 386**,** 266-273.

LESER, M. S., YANOVSKI, S. Z. & YANOVSKI, J. A. 2002. A low-fat intake and greater activity level are associated with lower weight regain 3 years after completing a very–low-calorie diet. *Journal of the American Dietetic Association,* 102**,** 1252-1256.

LESLIE, W. S., FORD, I., SATTAR, N., HOLLINGSWORTH, K. G., ADAMSON, A., SNIEHOTTA, F. F., MCCOMBIE, L., BROSNAHAN, N., ROSS, H., MATHERS, J. C., PETERS, C., THOM, G., BARNES, A., KEAN, S., MCILVENNA, Y., RODRIGUES, A., REHACKOVA, L., ZHYZHNEUSKAYA, S., TAYLOR, R. & LEAN, M. E. J. 2016. The Diabetes Remission Clinical Trial (DiRECT): protocol for a cluster randomised trial. *BMC Family Practice,* 17**,** 20.

LEVENE, A. P. & GOLDIN, R. D. 2012. The epidemiology, pathogenesis and histopathology of fatty liver disease. *Histopathology,* 61**,** 141-152.

LEVESQUE, E., WINTER, A., NOORAH, Z., DAURÈS, J.-P., LANDAIS, P., FERAY, C. & AZOULAY, D. 2017. Impact of acute-on-chronic liver failure on 90-day mortality following a first liver transplantation. *Liver International,* 37**,** 684-693.

LEVY, J. 1998. Evaluation of insulin sensitivity: the HOMA and CIGMA models. *Journees annuelles de diabetologie de l'Hotel-Dieu*.

LEWIS, M. C., PHILLIPS, M. L., SLAVOTINEK, J. P., KOW, L., THOMPSON, C. H. & TOOULI, J. 2006. Change in Liver Size and Fat Content after Treatment with Optifast® Very Low Calorie Diet. *Obesity Surgery,* 16**,** 697-701.

LI, J., ZOU, B., YEO, Y. H., FENG, Y., XIE, X., LEE, D. H., FUJII, H., WU, Y., KAM, L. Y., JI, F., LI, X., CHIEN, N., WEI, M., OGAWA, E., ZHAO, C., WU, X., STAVE, C. D., HENRY, L., BARNETT, S., TAKAHASHI, H., FURUSYO, N., EGUCHI, Y., HSU, Y.-C., LEE, T.-Y., REN, W., QIN, C., JUN, D. W., TOYODA, H., WONG, V. W.-S., CHEUNG, R., ZHU, Q. & NGUYEN, M. H. 2019. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999–2019: a systematic review and meta-analysis. *The Lancet Gastroenterology & Hepatology,* 4**,** 389-398.

LIM, E. L., HOLLINGSWORTH, K. G., ARIBISALA, B. S., CHEN, M. J., MATHERS, J. C. & TAYLOR, R. 2011. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia,* 54**,** 2506-14.

LIN, W.-Y., WU, C.-H., CHU, N.-F. & CHANG, C.-J. 2009. Efficacy and safety of very-low-calorie diet in Taiwanese: a multicenter randomized, controlled trial. *Nutrition,* 25**,** 1129-1136.

LIPS, M. A., DE GROOT, G. H., VAN KLINKEN, J. B., AARTS, E., BERENDS, F. J., JANSSEN, I. M., VAN RAMSHORST, B., VAN WAGENSVELD, B. A., SWANK, D. J. & VAN DIELEN, F. 2014. Calorie restriction is a major determinant of the short-term metabolic effects of gastric bypass surgery in obese type 2 diabetic patients. *Clinical endocrinology,* 80**,** 834-842.

LIU, Y.-L., REEVES, H. L., BURT, A. D., TINIAKOS, D., MCPHERSON, S., LEATHART, J. B. S., ALLISON, M. E. D., ALEXANDER, G. J., PIGUET, A.-C., ANTY, R., DONALDSON, P., AITHAL, G. P., FRANCQUE, S., VAN GAAL, L., CLEMENT, K., RATZIU, V., DUFOUR, J.-F.,

DAY, C. P., DALY, A. K. & ANSTEE, Q. M. 2014a. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nature Communications,* 5**,** 4309.

LIU, Y., DAI, M., BI, Y., XU, M., XU, Y., LI, M., WANG, T., HUANG, F., XU, B. & ZHANG, J. 2013. Active smoking, passive smoking, and risk of nonalcoholic fatty liver disease (NAFLD): a population-based study in China. *Journal of epidemiology***,** JE20120067.

LIU, Y. L., PATMAN, G. L., LEATHART, J. B. S., PIGUET, A. C., BURT, A. D., DUFOUR, J. F., DAY, C. P., DALY, A. K., REEVES, H. L. & ANSTEE, Q. M. 2014b. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *Journal of Hepatology,* 61**,** 75-81.

LOVE, J. G., MCKENZIE, J. S., NIKOKAVOURA, E. A., BROOM, J., ROLLAND, C. & JOHNSTON, K. L. 2016. The experiences of women with polycystic ovary syndrome on a very low-calorie diet. *International journal of women's health,* 8**,** 299.

LUDWIG, J., VIGGIANO, T. R., MCGILL, D. B. & OH, B. J. 1980. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc,* 55**,** 434-8.

LUUKKONEN, P. K., SÄDEVIRTA, S., ZHOU, Y., KAYSER, B., ALI, A., AHONEN, L., LALLUKKA, S., PELLOUX, V., GAGGINI, M., JIAN, C., HAKKARAINEN, A., LUNDBOM, N., GYLLING, H., SALONEN, A., OREŠIČ, M., HYÖTYLÄINEN, T., ORHO-MELANDER, M., RISSANEN, A., GASTALDELLI, A., CLÉMENT, K., HODSON, L. & YKI-JÄRVINEN, H. 2018. Saturated Fat Is More Metabolically Harmful for the Human Liver Than Unsaturated Fat or Simple Sugars. *Diabetes Care,* 41**,** 1732.

MACÍAS-RODRÍGUEZ, R. U., ILARRAZA-LOMELÍ, H., RUIZ-MARGÁIN, A., PONCE-DE-LEÓN-ROSALES, S., VARGAS-VORÁCKOVÁ, F., GARCÍA-FLORES, O., TORRE, A. & DUARTE-ROJO, A. 2016. Changes in Hepatic Venous Pressure Gradient Induced by Physical Exercise in Cirrhosis: Results of a Pilot Randomized Open Clinical Trial. *Clinical and translational gastroenterology* [Online], 7. Available: http://europepmc.org/abstract/MED/27415618

http://europepmc.org/articles/PMC5543482?pdf=render

http://europepmc.org/articles/PMC5543482

https://doi.org/10.1038/ctg.2016.38 [Accessed 2016/07//].

MACÍAS-RODRÍGUEZ, R. U., RUIZ-MARGÁIN, A., ROMÁN-CALLEJA, B. M., MORENO-TAVAREZ, E., WEBER-SANGRI, L., GONZÁLEZ-ARELLANO, M. F., FERNÁNDEZ-DEL-RIVERO, G. & RAMÍREZ-SOTO, K. 2019. Exercise prescription in patients with cirrhosis: Recommendations for clinical practice. *Revista de Gastroenterología de México (English Edition)*.

MALNICK, S., MILDINER, S. & NEUMAN, M. G. 2020. Obeticholic acid for treatment of NAFLD—A drug in search of a disease. *GastroHep,* 2**,** 133-137.

MARCHESINI, G., PETTA, S. & DALLE GRAVE, R. 2016. Diet, weight loss, and liver health in nonalcoholic fatty liver disease: Pathophysiology, evidence, and practice. *Hepatology,* 63**,** 2032-2043.

MARIN-ALEJANDRE, B. A., ABETE, I., CANTERO, I., RIEZU-BOJ, J. I., MILAGRO, F. I., MONREAL, J. I., ELORZ, M., HERRERO, J. I., BENITO-BOILLOS, A. & QUIROGA, J. 2019. Association between sleep disturbances and liver status in obese subjects with nonalcoholic fatty liver disease: A comparison with healthy controls. *Nutrients,* 11**,** 322.

MARKOVA, M., PIVOVAROVA, O., HORNEMANN, S., SUCHER, S., FRAHNOW, T., WEGNER, K., MACHANN, J., PETZKE, K. J., HIERHOLZER, J., LICHTINGHAGEN, R., HERDER, C., CARSTENSEN-KIRBERG, M., RODEN, M., RUDOVICH, N., KLAUS, S., THOMANN, R., SCHNEEWEISS, R., ROHN, S. & PFEIFFER, A. F. H. 2017. Isocaloric Diets High in Animal or Plant Protein Reduce Liver Fat and Inflammation in Individuals With Type 2 Diabetes. *Gastroenterology,* 152**,** 571-585.e8.

MARTIN, P., DIMARTINI, A., FENG, S., BROWN, B., FALLON, M. 2013. Evaluation for Liver Transplantation in Adults: 2013 Practise Guideline by the AASLD and the AST. AASLD.

MARTÍNEZ-GONZÁLEZ, M. Á., MARTINEZ, J. A., HU, F., GIBNEY, M. & KEARNEY, J. 1999. Physical inactivity, sedentary lifestyle and obesity in the European Union. *International journal of obesity,* 23**,** 1192-1201.

MARTINEZ-GONZALEZ, M. A., VARO, J. J., SANTOS, J. L., IRALA, J. D., GIBNEY, M., KEARNEY, J. & MARTINEZ, J. A. 2001. Prevalence of physical activity during leisure time in the European Union.

MASSY-WESTROPP, N. M., GILL, T. K., TAYLOR, A. W., BOHANNON, R. W. & HILL, C. L. 2011. Hand Grip Strength: age and gender stratified normative data in a population-based study. *BMC Research Notes,* 4**,** 127.

MASUDA, T., SHIRABE, K., IKEGAMI, T., HARIMOTO, N., YOSHIZUMI, T., SOEJIMA, Y., UCHIYAMA, H., IKEDA, T., BABA, H. & MAEHARA, Y. 2014. Sarcopenia is a prognostic factor in living donor liver transplantation. *Liver Transplantation,* 20**,** 401-407.

MATHUR, S., HORNBLOWER, E. & LEVY, R. D. 2009. Exercise Training Before and After Lung Transplantation. *The Physician and Sportsmedicine,* 37**,** 78-87.

MATTHEWS, C. E., HAGSTRÖMER, M., POBER, D. M. & BOWLES, H. R. 2012. Best practices for using physical activity monitors in population-based research. *Medicine and science in sports and exercise,* 44**,** S68.

MATTHEWS, D., HOSKER, J., RUDENSKI, A., NAYLOR, B., TREACHER, D. & TURNER, R. 1985. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia,* 28**,** 412-419.

MAZZOTTI, A., CALETTI, M. T., BRODOSI, L., DI DOMIZIO, S., FORCHIELLI, M. L., PETTA, S., BUGIANESI, E., BIANCHI, G. & MARCHESINI, G. 2018. An internet-based approach for lifestyle changes in patients with NAFLD: Two-year effects on weight loss and surrogate markers. *Journal of Hepatology,* 69**,** 1155-1163.

MCADAMS-DEMARCO, M. A., YING, H., VAN PILSUM RASMUSSEN, S., SCHRACK, J., HAUGEN, C. E., CHU, N. M., GONZÁLEZ FERNÁNDEZ, M., DESAI, N., WALSTON, J. D. & SEGEV, D. L. 2019. Prehabilitation prior to kidney transplantation: results from a pilot study. *Clinical transplantation,* 33**,** e13450.

MCCARTHY, M. M., FRANS, J. T., DAVEY, J. & CHYUN, D. A. 2017. Physical inactivity and cardiac events: An analysis of the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study. *Journal of clinical & translational endocrinology,* 9**,** 8-14.

MCPHERSON, S., HARDY, T., DUFOUR, J.-F., PETTA, S., ROMERO-GOMEZ, M., ALLISON, M., OLIVEIRA, C. P., FRANCQUE, S., VAN GAAL, L., SCHATTENBERG, J. M., TINIAKOS, D., BURT, A., BUGIANESI, E., RATZIU, V., DAY, C. P. & ANSTEE, Q. M. 2017. Age as a Confounding Factor for the Accurate Non-Invasive Diagnosis of Advanced NAFLD Fibrosis. *The American journal of gastroenterology,* 112**,** 740-751.

MCPHERSON, S., HARDY, T., HENDERSON, E., BURT, A. D., DAY, C. P. & ANSTEE, Q. M. 2015. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management. *Journal of Hepatology,* 62**,** 1148-1155.

MCPHERSON, S., JONSSON, J. R., COWIN, G. J., O'ROURKE, P., CLOUSTON, A. D., VOLP, A., HORSFALL, L., JOTHIMANI, D., FAWCETT, J., GALLOWAY, G. J., BENSON, M. & POWELL, E. E. 2009. Magnetic resonance imaging and spectroscopy accurately estimate the severity of steatosis provided the stage of fibrosis is considered. *Journal of Hepatology,* 51**,** 389-397.

MCPHERSON, S., STEWART, S. F., HENDERSON, E., BURT, A. D. & DAY, C. P. 2010. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut,* 59**,** 1265-1269.

MEAD, G. E., MORLEY, W., CAMPBELL, P., GREIG, C. A., MCMURDO, M. & LAWLOR, D. A. 2008. Exercise for depression. *Cochrane database of systematic reviews*.

MELLER, W., WELLE, N., SUTLEY, K. & THURBER, S. 2017. Depression and Liver Transplant Survival. *Psychosomatics,* 58**,** 64-68.

MEZA-JUNCO, J., MONTANO-LOZA, A. J., BARACOS, V. E., PRADO, C. M., BAIN, V. G., BEAUMONT, C., ESFANDIARI, N., LIEFFERS, J. R. & SAWYER, M. B. 2013. Sarcopenia as a prognostic index of nutritional status in concurrent cirrhosis and hepatocellular carcinoma. *Journal of clinical gastroenterology,* 47**,** 861-870.

MIGUELES, J. H., ROWLANDS, A. V., HUBER, F., SABIA, S. & VAN HEES, V. T. 2019. GGIR: A research community–driven open source R package for generating physical activity and sleep outcomes from multi-day raw accelerometer data. *Journal for the Measurement of Physical Behaviour,* 2**,** 188-196.

MOHAN, V., GOKULAKRISHNAN, K., DEEPA, R., SHANTHIRANI, C. & DATTA, M. 2005. Association of physical inactivity with components of metabolic syndrome and coronary artery disease—the Chennai Urban Population Study (CUPS no. 15). *Diabetic medicine,* 22**,** 1206-1211.

MONTANO–LOZA, A. J., MEZA–JUNCO, J., PRADO, C. M., LIEFFERS, J. R., BARACOS, V. E., BAIN, V. G. & SAWYER, M. B. 2012. Muscle wasting is associated with mortality in patients with cirrhosis. *Clinical Gastroenterology and Hepatology,* 10**,** 166-173. e1.

MONTEIRO, C. A., CONDE, W. L., MATSUDO, S. M., MATSUDO, V. R., BONSEÑOR, I. M. & LOTUFO, P. A. 2003. A descriptive epidemiology of leisure-time physical activity in Brazil, 1996-1997. *Revista Panamericana de Salud Publica,* 14**,** 246-254.

MORAN, J., GUINAN, E., MCCORMICK, P., LARKIN, J., MOCKLER, D., HUSSEY, J., MORIARTY, J. & WILSON, F. 2016. The ability of prehabilitation to influence postoperative outcome

after intra-abdominal operation: a systematic review and meta-analysis. *Surgery,* 160**,** 1189-1201.

MORENO, B., BELLIDO, D., SAJOUX, I., GODAY, A., SAAVEDRA, D., CRUJEIRAS, A. B. & CASANUEVA, F. F. 2014. Comparison of a very low-calorie-ketogenic diet with a standard low-calorie diet in the treatment of obesity. *Endocrine,* 47**,** 793-805.

MORRIS, E., AVEYARD, P., DYSON, P., NOREIK, M., BAILEY, C., FOX, R., JEROME, D., TAN, G. D. & JEBB, S. A. 2020. A food-based, low-energy, low-carbohydrate diet for people with type 2 diabetes in primary care: A randomized controlled feasibility trial. *Diabetes, Obesity and Metabolism,* 22**,** 512-520.

MOSS, M., WONG, C., MCMAHON, T., COUSINS, C. & JACKLYN, C. 1987. Qualitative and quantitative determination of porphyrins in urine, blood and feces. *Clinical Biochemistry,* 20**,** 290-291.

MURPHY, A. M., THOMAS, A., CRINION, S. J., KENT, B. D., TAMBUWALA, M. M., FABRE, A., PEPIN, J.-L., ROCHE, H. M., ARNAUD, C. & RYAN, S. 2017. Intermittent hypoxia in obstructive sleep apnoea mediates insulin resistance through adipose tissue inflammation. *European Respiratory Journal,* 49**,** 1601731.

MURPHY, S. L. 2009. Review of physical activity measurement using accelerometers in older adults: considerations for research design and conduct. *Preventive medicine,* 48**,** 108-114.

MURRAY, C. J. L., RICHARDS, M. A., NEWTON, J. N., FENTON, K. A., ANDERSON, H. R., ATKINSON, C., BENNETT, D., BERNABÉ, E., BLENCOWE, H., BOURNE, R., BRAITHWAITE, T., BRAYNE, C., BRUCE, N. G., BRUGHA, T. S., BURNEY, P., DHERANI, M., DOLK, H., EDMOND, K., EZZATI, M., FLAXMAN, A. D., FLEMING, T. D., FREEDMAN, G., GUNNELL, D., HAY, R. J., HUTCHINGS, S. J., OHNO, S. L., LOZANO, R., LYONS, R. A., MARCENES, W., NAGHAVI, M., NEWTON, C. R., PEARCE, N., POPE, D., RUSHTON, L., SALOMON, J. A., SHIBUYA, K., VOS, T., WANG, H., WILLIAMS, H. C., WOOLF, A. D., LOPEZ, A. D. & DAVIS, A. 2013. UK health performance: findings of the Global Burden of Disease Study 2010. *The Lancet,* 381**,** 997-1020.

MURRAY, K. F. & CARITHERS JR, R. L. 2005. AASLD practice guidelines: evaluation of the patient for liver transplantation. *Hepatology,* 41**,** 1407-1432.

MUSSO, G., CASSADER, M., ROSINA, F. & GAMBINO, R. 2012. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty

liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia,* 55**,** 885-904.

MYERS, R. P., POMIER-LAYRARGUES, G., KIRSCH, R., POLLETT, A., DUARTE-ROJO, A., WONG, D., BEATON, M., LEVSTIK, M., CROTTY, P. & ELKASHAB, M. 2012. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. *Hepatology,* 55**,** 199-208.

NAVARRO, J. E. J.-B., SANZ, J. L. G., DEL CASTILLO, J. M., IZQUIERDO, A. C. & RODRÍGUEZ, M. M. 2007. Motivational factors and physician advice for physical activity in older urban adults. *Journal of aging and physical activity,* 15**,** 241-256.

NETWORK, U. S. D. O. H. A. H. S. O. P. A. T. 2017. *National Data* [Online]. Available: https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/ [Accessed November 2020].

NEUSCHWANDER-TETRI, B. A., LOOMBA, R., SANYAL, A. J., LAVINE, J. E., VAN NATTA, M. L., ABDELMALEK, M. F., CHALASANI, N., DASARATHY, S., DIEHL, A. M., HAMEED, B., KOWDLEY, K. V., MCCULLOUGH, A., TERRAULT, N., CLARK, J. M., TONASCIA, J., BRUNT, E. M., KLEINER, D. E. & DOO, E. 2015. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *The Lancet,* 385**,** 956-965.

NEWTON, J. L., JONES, D. E., HENDERSON, E., KANE, L., WILTON, K., BURT, A. D. & DAY, C. P. 2008. Fatigue in non-alcoholic fatty liver disease (NAFLD) is significant and associates with inactivity and excessive daytime sleepiness but not with liver disease severity or insulin resistance. *Gut,* 57**,** 807-813.

NHS 2017. Annual Report on Liver Transplantation. *Report for 2016/2017.*

NHS. 2019. *Annual Report on Liver Transplantation* [Online]. Available: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/16782/nhsbt-liver-transplantation-annual-report-2018-19.pdf [Accessed September 2020 2020].

NICE 2016a. 6. Obesity Management for the Treatment of Type 2 Diabetes. *Diabetes Care,* 39**,** S47.

NICE. 2016b. *Lipid modification: cardiovascular risk assessment and the modification of blood lipids for the primary and secondary prevention of cardiovascular disease.* [Online]. National Institute for Health and Care Excellence. Available:

https://www.nice.org.uk/guidance/cg181/evidence/lipid-modification-update-full-guideline-pdf-243786637 [Accessed].

NICE. 2016c. *Non-alcoholic fatty liver disease (NAFLD): assessment and management* [Online]. NICE.org.uk. Available: https://www.nice.org.uk/guidance/ng49 [Accessed 22/05/2018 2018].

NISHIDA, Y., IDE, Y., OKADA, M., OTSUKA, T., EGUCHI, Y., OZAKI, I., TANAKA, K. & MIZUTA, T. 2017. Effects of home-based exercise and branched-chain amino acid supplementation on aerobic capacity and glycemic control in patients with cirrhosis. *Hepatology Research,* 47**,** E193-E200.

NORTH, T. C., MCCULLAGH, P. & TRAN, Z. V. 1990. Effect of exercise on depression. *Exercise and sport sciences reviews,* 18**,** 379-416.

NUNES, M. I. 2001. The relationship between quality of life and adherence to treatment. *Current Hypertension Reports,* 3**,** 462-465.

ÖSTBERG, A., WIKSTRAND, I. & BENGTSSON BOSTRÖM, K. 2011. Group treatment of obesity in primary care practice: a qualitative study of patients' perspectives. *Scandinavian journal of public health,* 39**,** 98-105.

OZE-FUKAI, A., FUJISAWA, T., SUGIMOTO, K., NOJIMA, K., SHINDO, N., SHIMOYOSHI, S., YOSHIKAWA, Y., SATO, Y., SHIMOMURA, I. & IKEGAMI, H. 2008. A novel mouse model for type 2 diabetes and non-alcoholic fatty liver disease: spontaneous amelioration of diabetes by augmented beta cell mass. *Endocrine journal***,** 0812010216-0812010216.

PAINTER, P., KRASNOFF, J., PAUL, S. M. & ASCHER, N. L. 2001. Physical activity and health-related quality of life in liver transplant recipients. *Liver transplantation,* 7**,** 213-219.

PAISEY, R., FROST, J., HARVEY, P., PAISEY, A., BOWER, L., PAISEY, R., TAYLOR, P. & BELKA, I. 2002. Five year results of a prospective very low calorie diet or conventional weight loss programme in type 2 diabetes. *Journal of Human Nutrition and Dietetics,* 15**,** 121-127.

PANG, J. X. Q., PRADHAN, F., ZIMMER, S., NIU, S., CROTTY, P., TRACEY, J., SCHNEIDER, C., HEITMAN, S. J., KAPLAN, G. G., SWAIN, M. G. & MYERS, R. P. 2014. The Feasibility and Reliability of Transient Elastography Using Fibroscan®: A Practice Audit of 2335 Examinations. *Canadian Journal of Gastroenterology and Hepatology,* 28**,** 952684.

PARRETTI, H. M., JEBB, S. A., JOHNS, D. J., LEWIS, A. L., CHRISTIAN-BROWN, A. M. & AVEYARD, P. 2016. Clinical effectiveness of very-low-energy diets in the management of weight loss: a systematic review and meta-analysis of randomized controlled trials. *Obes Rev,* 17**,** 225-34.

PATE, J., GUTIERREZ, J. A., FRENETTE, C. T., GOEL, A., KUMAR, S., MANCH, R. A., MENA, E. A., POCKROS, P. J., SATAPATHY, S. K., YIMAM, K. K. & GISH, R. G. 2019. Practical strategies for pruritus management in the obeticholic acid-treated patient with PBC: proceedings from the 2018 expert panel. *BMJ Open Gastroenterology,* 6**,** e000256.

PATEL, A. A., TORRES, D. M. & HARRISON, S. A. 2009. Effect of weight loss on nonalcoholic fatty liver disease. *Journal of clinical gastroenterology,* 43**,** 970-974.

PATRICK, D. L., BUSHNELL, D. M. & ROTHMAN, M. 2004. Performance of Two Self-Report Measures for Evaluating Obesity and Weight Loss*. *Obesity Research,* 12**,** 48-57.

PATRICK, L. & BUSHNELL, D. 2004. Obesity-specific patient reported outcomes: Obesity and weight loss quality of life (OWLQOL) and weight-related symptoms measure (WRSM). *User's Manual and Scoring Diskette for United States Version. Washington, Seattle University of Washington***,** 1-45.

PAVELA, G., HARMAN, T., CARDEL, M. I. & LEE, A. 2020. Obesity and Socioeconomic Status. *Handbook of Eating and Drinking: Interdisciplinary Perspectives***,** 805-822.

PEPPARD, P. E., YOUNG, T., PALTA, M., DEMPSEY, J. & SKATRUD, J. 2000. Longitudinal study of moderate weight change and sleep-disordered breathing. *Jama,* 284**,** 3015-3021.

PERRY, R. J., PENG, L., CLINE, G. W., WANG, Y., RABIN-COURT, A., SONG, J. D., ZHANG, D., ZHANG, X.-M., NOZAKI, Y. & DUFOUR, S. 2018. Mechanisms by which a very-low-calorie diet reverses hyperglycemia in a rat model of type 2 diabetes. *Cell metabolism,* 27**,** 210-217. e3.

PERRY, R. J., SAMUEL, V. T., PETERSEN, K. F. & SHULMAN, G. I. 2014. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature,* 510**,** 84-91.

PETERSEN, L., SCHNOHR, P. & SØRENSEN, T. I. A. 2004. Longitudinal study of the long-term relation between physical activity and obesity in adults. *International Journal of Obesity,* 28**,** 105-112.

PICKETT, K. E. & PEARL, M. 2001. Multilevel analyses of neighbourhood socioeconomic context and health outcomes: a critical review. *Journal of Epidemiology & Community Health,* 55**,** 111-122.

PLASQUI, G. & WESTERTERP, K. R. 2007. Physical activity assessment with accelerometers: an evaluation against doubly labeled water. *Obesity,* 15**,** 2371-2379.

POIRIER, P., HERNANDEZ, T. L., WEIL, K. M., SHEPARD, T. J. & ECKEL, R. H. 2003. Impact of diet-induced weight loss on the cardiac autonomic nervous system in severe obesity. *Obesity research,* 11**,** 1040-1047.

POLONSKY, W. H. & FISHER, L. 2015. When does personalized feedback make a difference? A narrative review of recent findings and their implications for promoting better diabetes self-care. *Current diabetes reports,* 15**,** 50.

POORTINGA, W. 2007. The prevalence and clustering of four major lifestyle risk factors in an English adult population. *Preventive Medicine,* 44**,** 124-128.

PRASHANTH, M., GANESH, H. K., VIMA, M. V., JOHN, M., BANDGAR, T., JOSHI, S. R., SHAH, S. R., RATHI, P. M., JOSHI, A. S., THAKKAR, H., MENON, P. S. & SHAH, N. S. 2009. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *J Assoc Physicians India,* 57**,** 205-10.

PRENTIS, J. M., MANAS, D. M., TRENELL, M. I., HUDSON, M., JONES, D. J. & SNOWDEN, C. P. 2012. Submaximal cardiopulmonary exercise testing predicts 90-day survival after liver transplantation. *Liver Transpl,* 18**,** 152-9.

PROMRAT, K., KLEINER, D. E., NIEMEIER, H. M., JACKVONY, E., KEARNS, M., WANDS, J. R., FAVA, J. L. & WING, R. R. 2010. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology,* 51**,** 121-129.

RANGARI, A. S., SAMAL, S. & SAMAL, S. 2019. Measurement of VO2 in sedentary healthy females: A normative study. *Age (year),* 22**,** 2.7.

RATZIU, V., HARRISON, S. A., FRANCQUE, S., BEDOSSA, P., LEHERT, P., SERFATY, L., ROMERO-GOMEZ, M., BOURSIER, J., ABDELMALEK, M., CALDWELL, S., DRENTH, J., ANSTEE, Q. M., HUM, D., HANF, R., ROUDOT, A., MEGNIEN, S., STAELS, B. & SANYAL, A. 2016. Elafibranor, an Agonist of the Peroxisome Proliferator-Activated Receptor-alpha and -delta, Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening. *Gastroenterology,* 150**,** 1147-1159.e5.

RCORE, T. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

RECTOR, R. S. & THYFAULT, J. P. 2011. Does physical inactivity cause nonalcoholic fatty liver disease? *Journal of applied physiology,* 111**,** 1828-1835.

REHACKOVA, L., ARAÚJO-SOARES, V., ADAMSON, A., STEVEN, S., TAYLOR, R. & SNIEHOTTA, F. 2017. Acceptability of a very-low-energy diet in Type 2 diabetes: patient experiences and behaviour regulation. *Diabetic Medicine,* 34**,** 1554-1567.

REHACKOVA, L., ARAÚJO-SOARES, V., STEVEN, S., ADAMSON, A., TAYLOR, R. & SNIEHOTTA, F. 2020. Behaviour change during dietary Type 2 diabetes remission: a longitudinal qualitative evaluation of an intervention using a very low energy diet. *Diabetic Medicine,* 37**,** 953-962.

REHACKOVA, L., ARNOTT, B., ARAUJO-SOARES, V., ADAMSON, A. A., TAYLOR, R. & SNIEHOTTA, F. F. 2016. Efficacy and acceptability of very low energy diets in overweight and obese people with Type 2 diabetes mellitus: a systematic review with meta-analyses. *Diabetic Medicine,* 33**,** 580-591.

REUTRAKUL, S. & VAN CAUTER, E. 2018. Sleep influences on obesity, insulin resistance, and risk of type 2 diabetes. *Metabolism,* 84**,** 56-66.

RIKLI, R. E. 2000. Reliability, validity, and methodological issues in assessing physical activity in older adults. *Research quarterly for exercise and sport,* 71**,** 89-96.

ROGAL, S. S., MANKANEY, G., UDAWATTA, V., CHINMAN, M., GOOD, C. B., ZICKMUND, S., BIELEFELDT, K., CHIDI, A., JONASSAINT, N., JAZWINSKI, A., SHAIKH, O., HUGHES, C., FONTES, P., HUMAR, A. & DIMARTINI, A. 2016. Pre-Transplant Depression Is Associated with Length of Hospitalization, Discharge Disposition, and Survival after Liver Transplantation. *PLOS ONE,* 11**,** e0165517.

ROMÁN, E., GARCÍA-GALCERÁN, C., TORRADES, T., HERRERA, S., MARÍN, A., DOÑATE, M., ALVARADO-TAPIAS, E., MALOUF, J., NÁCHER, L., SERRA-GRIMA, R., GUARNER, C., CORDOBA, J. & SORIANO, G. 2016. Effects of an Exercise Programme on Functional Capacity, Body Composition and Risk of Falls in Patients with Cirrhosis: A Randomized Clinical Trial. *PLOS ONE,* 11**,** e0151652.

ROMÁN, E., TORRADES, M. T., NADAL, M. J., CÁRDENAS, G., NIETO, J. C., VIDAL, S., BASCUÑANA, H., JUÁREZ, C., GUARNER, C. & CÓRDOBA, J. 2014. Randomized pilot study: effects of an exercise programme and leucine supplementation in patients with cirrhosis. *Digestive diseases and sciences,* 59**,** 1966-1975.

ROMERO-GÓMEZ, M., ZELBER-SAGI, S. & TRENELL, M. 2017. Treatment of NAFLD with diet, physical activity and exercise. *Journal of hepatology,* 67**,** 829-846.

RUTH, M., WENDY, B. & CATRINE, T.-L. 2006. But What About Swimming and Cycling? How to "Count" Non-Ambulatory Activity When Using Pedometers to Assess Physical Activity. *Journal of Physical Activity and Health,* 3**,** 257-266.

RYAN, M. C., ITSIOPOULOS, C., THODIS, T., WARD, G., TROST, N., HOFFERBERTH, S., O'DEA, K., DESMOND, P. V., JOHNSON, N. A. & WILSON, A. M. 2013. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. *Journal of hepatology,* 59**,** 138-143.

RYTTIG, K., FLATEN, H. & RÖSSNER, S. 1997. Long-term effects of a very low calorie diet (Nutrilett®) in obesity treatment. A prospective, randomized, comparison between VLCD and a hypocaloric diet+ behavior modification and their combination. *International Journal of Obesity & Related Metabolic Disorders,* 21.

RYU, S., CHANG, Y., JUNG, H.-S., YUN, K. E., KWON, M.-J., CHOI, Y., KIM, C.-W., CHO, J., SUH, B.-S. & CHO, Y. K. 2015. Relationship of sitting time and physical activity with non-alcoholic fatty liver disease. *Journal of hepatology,* 63**,** 1229-1237.

SAADEH, S., YOUNOSSI, Z. M., REMER, E. M., GRAMLICH, T., ONG, J. P., HURLEY, M., MULLEN, K. D., COOPER, J. N. & SHERIDAN, M. J. 2002. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology,* 123**,** 745-50.

SABIA, S., VAN HEES, V. T., SHIPLEY, M. J., TRENELL, M. I., HAGGER-JOHNSON, G., ELBAZ, A., KIVIMAKI, M. & SINGH-MANOUX, A. 2014. Association between questionnaire-and accelerometer-assessed physical activity: the role of sociodemographic factors. *American journal of epidemiology,* 179**,** 781-790.

SALLIS, J. F. & SAELENS, B. E. 2000. Assessment of physical activity by self-report: status, limitations, and future directions. *Research quarterly for exercise and sport,* 71**,** 1-14.

SANTORO, N., ZHANG, C. K., ZHAO, H., PAKSTIS, A. J., KIM, G., KURSAWE, R., DYKAS, D. J., BALE, A. E., GIANNINI, C., PIERPONT, B., SHAW, M. M., GROOP, L. & CAPRIO, S. 2012. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. *Hepatology,* 55**,** 781-789.

SANYAL, A. J., CHALASANI, N., KOWDLEY, K. V., MCCULLOUGH, A., DIEHL, A. M., BASS, N. M., NEUSCHWANDER-TETRI, B. A., LAVINE, J. E., TONASCIA, J., UNALP, A., VAN NATTA, M., CLARK, J., BRUNT, E. M., KLEINER, D. E., HOOFNAGLE, J. H. & ROBUCK, P. R. 2010. Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis. *New England Journal of Medicine,* 362**,** 1675-1685.

SARIS, W. H. M. 2001. Very-Low-Calorie Diets and Sustained Weight Loss. *Obesity Research,* 9**,** 295S-301S.

SAVERYMUTTU, S., JOSEPH, A. & MAXWELL, J. 1986. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *Br Med J (Clin Res Ed),* 292**,** 13-15.

SCHLENKER, B. R. & LEARY, M. R. 1982. Social anxiety and self-presentation: A conceptualization model. *Psychological bulletin,* 92**,** 641.

SCHWENGER, K. J., FISCHER, S. E., JACKSON, T. D., OKRAINEC, A. & ALLARD, J. P. 2018. Non-alcoholic fatty liver disease in morbidly obese individuals undergoing bariatric surgery: prevalence and effect of the pre-bariatric very low calorie diet. *Obesity surgery,* 28**,** 1109-1116.

SCRAGG, J., AVERY, L., CASSIDY, S., TAYLOR, G., HAIGH, L., BOYLE, M., TRENELL, M. I., ANSTEE, Q. M., MCPHERSON, S. & HALLSWORTH, K. 2020. Feasibility of a Very Low Calorie Diet to Achieve a Sustainable 10% Weight Loss in Patients With Nonalcoholic Fatty Liver Disease. *Clinical and Translational Gastroenterology,* 11**,** e00231.

SHAKER, M., TABBAA, A., ALBELDAWI, M. & ALKHOURI, N. 2014. Liver transplantation for nonalcoholic fatty liver disease: new challenges and new opportunities. *World journal of gastroenterology,* 20**,** 5320-5330.

SHARMA, P., SCHAUBEL DOUGLAS, E., GOODRICH NATHAN, P. & MERION ROBERT, M. 2015. Serum sodium and survival benefit of liver transplantation. *Liver Transplantation,* 21**,** 308-313.

SHEN, J., WONG, G. L. H., CHAN, H. L. Y., CHAN, H. Y., YEUNG, D. K. W., CHAN, R. S. M., CHIM, A. M. L., CHAN, A. W. H., CHOI, P. C. L., WOO, J., CHU, W. C. W. & WONG, V. W. S. 2014. PNPLA3 gene polymorphism accounts for fatty liver in community subjects without metabolic syndrome. *Alimentary Pharmacology & Therapeutics,* 39**,** 532-539.

SHEPHARD, R. J. 2003. Limits to the measurement of habitual physical activity by questionnaires. *British journal of sports medicine,* 37**,** 197-206.

SHIGEMATSU, R., UENO, L. M., NAKAGAICHI, M., NHO, H. & TANAKA, K. 2004. Rate of perceived exertion as a tool to monitor cycling exercise intensity in older adults. *Journal of Aging and Physical Activity,* 12**,** 3-9.

SHIROMA, E. J., COOK, N. R., MANSON, J. E., BURING, J. E., RIMM, E. B. & LEE, I.-M. 2015. Comparison of self-reported and accelerometer-assessed physical activity in older women. *PloS one,* 10**,** e0145950.

SIEDEL, J., SCHIEFER, S., ROSSENEU, M., BERGEAUD, R., DE KEERSGIETER, W., PAUTZ, B., VINAIMONT, N. & ZIEGENHORN, J. 1988. Immunoturbidimetric method for routine determinations of apolipoproteins AI, A-II, and B in normo-and hyperlipemic sera compared with immunonephelometry. *Clinical chemistry,* 34**,** 1821-1825.

SIEDEL, J., SCHMUCK, R., STAEPELS, J. & TOWN, M. 1993. Long term stable, liquid ready-to-use monoreagent for the enzymatic assay of serum or plasma triglycerides (GPO-PAP method). AACC meeting abstract 34. *Clin Chem,* 39**,** 1127.

SIM, J. & LEWIS, M. 2012. The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency. *Journal of Clinical Epidemiology,* 65**,** 301-308.

SINGH, H., POLLOCK, R., UHANOVA, J., KRYGER, M., HAWKINS, K. & MINUK, G. Y. 2005. Symptoms of obstructive sleep apnea in patients with nonalcoholic fatty liver disease. *Digestive diseases and sciences,* 50**,** 2338-2343.

SKYTTE, M. J., SAMKANI, A., PETERSEN, A. D., THOMSEN, M. N., ASTRUP, A., CHABANOVA, E., FRYSTYK, J., HOLST, J. J., THOMSEN, H. S., MADSBAD, S., LARSEN, T. M., HAUGAARD, S. B. & KRARUP, T. 2019. A carbohydrate-reduced high-protein diet improves HbA1c and liver fat content in weight stable participants with type 2 diabetes: a randomised controlled trial. *Diabetologia,* 62**,** 2066-2078.

SMART, N. & STEELE, M. 2011. Exercise training in haemodialysis patients: a systematic review and meta-analysis. *Nephrology (Carlton),* 16**,** 626-32.

SMITH, B. W. & ADAMS, L. A. 2011. Non-alcoholic fatty liver disease. *Critical Reviews in Clinical Laboratory Sciences,* 48**,** 97-113.

SMITS MARK, M., IOANNOU GEORGE, N., BOYKO EDWARD, J. & UTZSCHNEIDER KRISTINA, M. 2013. Non-alcoholic fatty liver disease as an independent manifestation of the metabolic syndrome: Results of a US national survey in three ethnic groups. *Journal of Gastroenterology and Hepatology,* 28**,** 664-670.

SNEL, M., SLEDDERING, M. A., VD PEIJL, I. D., ROMIJN, J. A., PIJL, H., MEINDERS, A. E. & JAZET, I. M. 2012. Quality of life in type 2 diabetes mellitus after a very low calorie diet and exercise. *European Journal of Internal Medicine,* 23**,** 143-149.

SPENGLER, E. K., O'LEARY, J. G., TE, H. S., ROGAL, S., PILLAI, A. A., AL-OSAIMI, A., DESAI, A., FLEMING, J. N., GANGER, D., SEETHARAM, A., TSOULFAS, G., MONTENOVO, M. & LAI, J. C. 2017. Liver Transplantation in the Obese Cirrhotic Patient. *Transplantation,* 101**,** 2288-2296.

ST. GEORGE, A., BAUMAN, A., JOHNSTON, A., FARRELL, G., CHEY, T. & GEORGE, J. 2009. Effect of a lifestyle intervention in patients with abnormal liver enzymes and metabolic risk factors. *Journal of Gastroenterology and Hepatology,* 24**,** 399-407.

STERLING, R. K., LISSEN, E., CLUMECK, N., SOLA, R., CORREA, M. C., MONTANER, J., S. SULKOWSKI, M., TORRIANI, F. J., DIETERICH, D. T. & THOMAS, D. L. 2006. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology,* 43**,** 1317-1325.

STEVEN, S., HOLLINGSWORTH, K. G., AL-MRABEH, A., AVERY, L., ARIBISALA, B., CASLAKE, M. & TAYLOR, R. 2016. Very Low-Calorie Diet and 6 Months of Weight Stability in Type 2 Diabetes: Pathophysiological Changes in Responders and Nonresponders. *Diabetes Care,* 39**,** 808.

STUNKARD, A. J. & SORENSEN, T. I. 1993. Obesity and socioeconomic status--a complex relation. Mass Medical Soc.

SUN, W. H., SONG, M. Q., JIANG, C. Q., XIN, Y. N., MA, J. L., LIU, Y. X., MA, L., LIN, Z. H., LI, C. Y., LIU, L., ZHANG, M., CHU, L. L., JIANG, X. J., WAN, Q., ZHOU, L., REN, R. & MENG, L. F. 2012. Lifestyle intervention in non-alcoholic fatty liver disease in Chengyang District, Qingdao, China. *World J Hepatol,* 4**,** 224-30.

SURATT, P. M., MCTIER, R. F., FINDLEY, L. J., POHL, S. & WILHOIT, S. C. 1992. Effect of very-low-calorie diets with weight loss on obstructive sleep apnea. *The American journal of clinical nutrition,* 56**,** 182S-184S.

SWAIN, D. P., BRAWNER, C. A. & MEDICINE, A. C. O. S. 2014. *ACSM's resource manual for guidelines for exercise testing and prescription*, Wolters Kluwer Health/Lippincott Williams & Wilkins.

SYSKO, R., DEVLIN, M. J., HILDEBRANDT, T. B., BREWER, S. K., ZITSMAN, J. L. & WALSH, B. T. 2012. Psychological outcomes and predictors of initial weight loss outcomes among severely obese adolescents receiving laparoscopic adjustable gastric banding. *The Journal of clinical psychiatry,* 73**,** 1351-1357.

SZASZ, G. 1976. Reaction-rate method for gamma-glutamyltransferase activity in serum. *Clinical chemistry,* 22**,** 2051-2055.

SZCZEPANIAK, L. S., NURENBERG, P., LEONARD, D., BROWNING, J. D., REINGOLD, J. S., GRUNDY, S., HOBBS, H. H. & DOBBINS, R. L. 2005. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *American Journal of Physiology-Endocrinology and Metabolism,* 288**,** E462-E468.

TANDON, P., ISMOND, K. P., RIESS, K., DUARTE-ROJO, A., AL-JUDAIBI, B., DUNN, M. A., HOLMAN, J., HOWES, N., HAYKOWSKY, M. J. F., JOSBENO, D. A. & MCNEELY, M. 2018. Exercise in cirrhosis: Translating evidence and experience to practice. *Journal of Hepatology,* 69**,** 1164-1177.

TAPPER, E. B., FINKELSTEIN, D., MITTLEMAN, M. A., PIATKOWSKI, G. & LAI, M. 2015. Standard assessments of frailty are validated predictors of mortality in hospitalized patients with cirrhosis. *Hepatology,* 62**,** 584-590.

TAYLOR, R. 2013. Type 2 diabetes: etiology and reversibility. *Diabetes care,* 36**,** 1047-1055.

TAYLOR, R. S., TAYLOR, R. J., BAYLISS, S., HAGSTROM, H., NASR, P., SCHATTENBERG, J. M., ISHIGAMI, M., TOYODA, H., WAI-SUN WONG, V., PELEG, N., SHLOMAI, A., SEBASTIANI, G., SEKO, Y., BHALA, N., YOUNOSSI, Z. M., ANSTEE, Q. M., MCPHERSON, S., NEWSOME, P. N., EKSTEDT, M., STÅL, P., HULTCRANTZ, R. & KECHAGIAS, S. 2020. Association Between Fibrosis Stage and Outcomes of Patients with Non-Alcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis. *Gastroenterology*.

TEMMERMAN, J. C. & FRIEDMAN, A. N. 2013. Very low calorie ketogenic weight reduction diet in patients with cirrhosis: a case series. *Nutrition & Diabetes,* 3**,** e95.

TENDLER, D., LIN, S., YANCY, W. S., JR., MAVROPOULOS, J., SYLVESTRE, P., ROCKEY, D. C. & WESTMAN, E. C. 2007. The effect of a low-carbohydrate, ketogenic diet on nonalcoholic fatty liver disease: a pilot study. *Dig Dis Sci,* 52**,** 589-93.

THOM, G., MESSOW, C. M., LESLIE, W. S., BARNES, A. C., BROSNAHAN, N., MCCOMBIE, L., AL-MRABEH, A., ZHYZHNEUSKAYA, S., WELSH, P., SATTAR, N., TAYLOR, R. & LEAN, M. E. J. 2020a. Predictors of type 2 diabetes remission in the Diabetes Remission Clinical Trial (DiRECT). *Diabetic Medicine,* n/a**,** e14395.

THOM, G., MESSOW, C. M., LESLIE, W. S., BARNES, A. C., BROSNAHAN, N., MCCOMBIE, L., AL-MRABEH, A., ZHYZHNEUSKAYA, S., WELSH, P., SATTAR, N., TAYLOR, R. & LEAN, M.

E. J. 2020b. Predictors of type 2 diabetes remission in the Diabetes Remission Clinical Trial (DiRECT). *Diabetic Medicine,* n/a.

THOMA, C., DAY, C. P. & TRENELL, M. I. 2012. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. *Journal of hepatology,* 56**,** 255-266.

THOMAS, J. G., BOND, D. S., PHELAN, S., HILL, J. O. & WING, R. R. 2014. Weight-loss maintenance for 10 years in the National Weight Control Registry. *American journal of preventive medicine,* 46**,** 17-23.

TOBKES, A. I. & NORD, J. 1995. Liver biopsy: review of methodology and complications. *Digestive diseases,* 13**,** 267-274.

TOMASONE, J., KAUFFELDT, K., MORGAN, T., MAGOR, K., LATIMER-CHEUNG, A., FAULKNER, G., ROSS-WHITE, A., POITRAS, V., KHO, M. & ROSS, R. 2020. Dissemination and implementation of national physical activity, sedentary behaviour, and/or sleep guidelines among community-dwelling adults aged 18 years and older: a systematic scoping review and suggestions for future reporting and research. *Applied Physiology, Nutrition, and Metabolism,* 45**,** S258-S283.

TONG, A., SAINSBURY, P. & CRAIG, J. 2007. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. *Int J Qual Health Care,* 19**,** 349-57.

TOWNSEND, P., PHILLIMORE, P. & BEATTIE, A. 1988. *Health and deprivation: inequality and the North*, Routledge.

TROVATO, G. M., CATALANO, D., MARTINES, G. F., PIRRI, C. & TROVATO, F. M. 2013. Western dietary pattern and sedentary life: independent effects of diet and physical exercise intensity on NAFLD. *The American Journal of Gastroenterology,* 108**,** 1932.

TSAI, A. G. & WADDEN, T. A. 2006. The Evolution of Very-Low-Calorie Diets: An Update and Meta-analysis. *Obesity,* 14**,** 1283-1293.

UENO, T., SUGAWARA, H., SUJAKU, K., HASHIMOTO, O., TSUJI, R., TAMAKI, S., TORIMURA, T., INUZUKA, S., SATA, M. & TANIKAWA, K. 1997. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *Journal of Hepatology,* 27**,** 103-107.

VAINSHELBOIM, B., MÜLLER, J., LIMA, R. M., NEAD, K. T., CHESTER, C., CHAN, K., KOKKINOS, P. & MYERS, J. 2017. Cardiorespiratory fitness, physical activity and cancer mortality in men. *Preventive medicine,* 100**,** 89-94.

VALLET-PICHARD, A., MALLET, V., NALPAS, B., VERKARRE, V., NALPAS, A., DHALLUIN-VENIER, V., FONTAINE, H. & POL, S. 2007. FIB-4: An inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology,* 46**,** 32-36.

VALLET-PICHARD, A., MALLET, V., NALPAS, B., VERKARRE, V., NALPAS, A., DHALLUIN-VENIER, V., FONTAINE, H. & POL, S. 2007. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology,* 46**,** 32-36.

VAN CAUTER, E. 2011. Sleep disturbances and insulin resistance. *Diabetic Medicine,* 28**,** 1455-1462.

VAN DER MEER, S., ZWERINK, M., VAN BRUSSEL, M., VAN DER VALK, P., WAJON, E. & VAN DER PALEN, J. 2012. Effect of outpatient exercise training programmes in patients with chronic heart failure: a systematic review. *Eur J Prev Cardiol,* 19**,** 795-803.

VAN DYCK, D., CERIN, E., DE BOURDEAUDHUIJ, I., HINCKSON, E., REIS, R. S., DAVEY, R., SARMIENTO, O. L., MITAS, J., TROELSEN, J. & MACFARLANE, D. 2015. International study of objectively measured physical activity and sedentary time with body mass index and obesity: IPEN adult study. *International Journal of Obesity,* 39**,** 199-207.

VAN HEES, V. T., FANG, Z., LANGFORD, J., ASSAH, F., MOHAMMAD, A., DA SILVA, I. C., TRENELL, M. I., WHITE, T., WAREHAM, N. J. & BRAGE, S. 2014. Autocalibration of accelerometer data for free-living physical activity assessment using local gravity and temperature: an evaluation on four continents. *Journal of Applied Physiology,* 117**,** 738-744.

VAN HEES, V. T., GORZELNIAK, L., LEON, E. C. D., EDER, M., PIAS, M., TAHERIAN, S., EKELUND, U., RENSTRÖM, F., FRANKS, P. W. & HORSCH, A. 2013. Separating movement and gravity components in an acceleration signal and implications for the assessment of human daily physical activity. *PloS one,* 8**,** e61691.

VAN HEES, V. T., SABIA, S., ANDERSON, K. N., DENTON, S. J., OLIVER, J., CATT, M., ABELL, J. G., KIVIMÄKI, M., TRENELL, M. I. & SINGH-MANOUX, A. 2015. A novel, open access

method to assess sleep duration using a wrist-worn accelerometer. *PloS one,* 10**,** e0142533.

VAN VUGT, J., LEVOLGER, S., DE BRUIN, R., VAN ROSMALEN, J., METSELAAR, H. & IJZERMANS, J. 2016. Systematic review and meta-analysis of the impact of computed tomography–assessed skeletal muscle mass on outcome in patients awaiting or undergoing liver transplantation. *American Journal of Transplantation,* 16**,** 2277-2292.

VARTANIAN, L. R., WHARTON, C. M. & GREEN, E. B. 2012. Appearance vs. health motives for exercise and for weight loss. *Psychology of Sport and Exercise,* 13**,** 251-256.

VILAR-GOMEZ, E., MARTINEZ-PEREZ, Y., CALZADILLA-BERTOT, L., TORRES-GONZALEZ, A., GRA-ORAMAS, B., GONZALEZ-FABIAN, L., FRIEDMAN, S. L., DIAGO, M. & ROMERO-GOMEZ, M. 2015. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology,* 149**,** 367-378.e5.

VILJANEN, A. P. M., IOZZO, P., BORRA, R., KANKAANPÄÄ, M., KARMI, A., LAUTAMÄKI, R., JÄRVISALO, M., PARKKOLA, R., RÖNNEMAA, T., GUIDUCCI, L., LEHTIMÄKI, T., RAITAKARI, O. T., MARI, A. & NUUTILA, P. 2009. Effect of Weight Loss on Liver Free Fatty Acid Uptake and Hepatic Insulin Resistance. *The Journal of Clinical Endocrinology & Metabolism,* 94**,** 50-55.

VOS, B., MORENO, C., NAGY, N., FERY, F., CNOP, M., VEREERSTRAETEN, P., DEVIERE, J. & ADLER, M. 2011. Lean non-alcoholic fatty liver disease (Lean-NAFLD): a major cause of cryptogenic liver disease. *Acta Gastroenterol Belg,* 74**,** 389-94.

WALLACE, T., LEVY, J. & MATTHEWS, D. 2004. An increase in insulin sensitivity and basal beta-cell function in diabetic subjects treated with pioglitazone in a placebo-controlled randomized study. *Diabetic medicine,* 21**,** 568-576.

WALLEN, M. P., SKINNER, T. L., PAVEY, T. G., HALL, A., MACDONALD, G. A. & COOMBES, J. S. 2016. Safety, adherence and efficacy of exercise training in solid-organ transplant candidates: A systematic review. *Transplantation Reviews,* 30**,** 218-226.

WASSERMAN, K., HANSEN, J. E., SUE, D. Y., STRINGER, W. W. & WHIPP, B. J. 2005. Principles of exercise testing and interpretation: including pathophysiology and clinical applications. *Medicine & Science in Sports & Exercise,* 37**,** 1249.

WATT, K. D. S., PEDERSEN, R. A., KREMERS, W. K., HEIMBACH, J. K. & CHARLTON, M. R. 2010. Evolution of Causes and Risk Factors for Mortality Post-Liver Transplant: Results of

the NIDDK Long-Term Follow-Up Study. *American Journal of Transplantation,* 10**,** 1420-1427.

WEINSIER, R. L., HUNTER, G. R., ZUCKERMAN, P. A., REDDEN, D. T., DARNELL, B. E., LARSON, D. E., NEWCOMER, B. R. & GORAN, M. I. 2000. Energy expenditure and free-living physical activity in black and white women: comparison before and after weight loss. *The American journal of clinical nutrition,* 71**,** 1138-1146.

WIELAND, A. C., METTLER, P., MCDERMOTT, M. T., CRANE, L. A., CICUTTO, L. C. & BAMBHA, K. M. 2015. Low awareness of nonalcoholic fatty liver disease among patients at high metabolic risk. *Journal of clinical gastroenterology,* 49**,** e6-e10.

WILLIAMS, A. M., WAITS, S. & ENGLESBE, M. J. 2015. The Importance of Prehabilitation in Liver Transplantation. *Current Transplantation Reports,* 2**,** 312-315.

WILLIAMS, F. R., VALLANCE, A., FAULKNER, T., TOWEY, J., DURMAN, S., KYTE, D., ELSHARKAWY, A. M., PERERA, T., HOLT, A., FERGUSON, J., LORD, J. M. & ARMSTRONG, M. J. 2019. Home-Based Exercise in Patients Awaiting Liver Transplantation: A Feasibility Study. *Liver Transplantation,* 25**,** 995-1006.

WILLIAMS, F. R., VALLANCE, A., FAULKNER, T., TOWEY, J., KYTE, D., DURMAN, S., JOHNSON, J., HOLT, A., PERERA, M. T., FERGUSON, J. & ARMSTRONG, M. J. 2018. Home-based exercise therapy in patients awaiting liver transplantation: protocol for an observational feasibility trial. *BMJ Open,* 8.

WILLIAMS, K. V., MULLEN, M. L., KELLEY, D. E. & WING, R. R. 1998. The effect of short periods of caloric restriction on weight loss and glycemic control in type 2 diabetes. *Diabetes care,* 21**,** 2-8.

WING, R. R., BLAIR, E., MARCUS, M., EPSTEIN, L. H. & HARVEY, J. 1994. Year-long weight loss treatment for obese patients with type II diabetes: does including an intermittent very-low-calorie diet improve outcome? *The American journal of medicine,* 97**,** 354-362.

WING, R. R., LANG, W., WADDEN, T. A., SAFFORD, M., KNOWLER, W. C., BERTONI, A. G., HILL, J. O., BRANCATI, F. L., PETERS, A. & WAGENKNECHT, L. 2011. Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. *Diabetes care,* 34**,** 1481-1486.

WING, R. R., MARCUS, M. D., SALATA, R., EPSTEIN, L. H., MIASKIEWICZ, S. & BLAIR, E. H. 1991. Effects of a very-low-calorie diet on long-term glycemic control in obese type 2 diabetic subjects. *Archives of Internal Medicine,* 151**,** 1334-1340.

WING, R. R. & PHELAN, S. 2005. Long-term weight loss maintenance–. *The American journal of clinical nutrition,* 82**,** 222S-225S.

WONG, R. J., AGUILAR, M., CHEUNG, R., PERUMPAIL, R. B., HARRISON, S. A., YOUNOSSI, Z. M. & AHMED, A. 2015. Nonalcoholic Steatohepatitis Is the Second Leading Etiology of Liver Disease Among Adults Awaiting Liver Transplantation in the United States. *Gastroenterology,* 148**,** 547-555.

WONG, V. W.-S., CHAN, R. S.-M., WONG, G. L.-H., CHEUNG, B. H.-K., CHU, W. C.-W., YEUNG, D. K.-W., CHIM, A. M.-L., LAI, J. W.-Y., LI, L. S., SEA, M. M.-M., CHAN, F. K.-L., SUNG, J. J.-Y., WOO, J. & CHAN, H. L.-Y. 2013. Community-based lifestyle modification programme for non-alcoholic fatty liver disease: A randomized controlled trial. *Journal of Hepatology,* 59**,** 536-542.

WONG, V. W.-S., WONG, G. L.-H., CHAN, R. S.-M., SHU, S. S.-T., CHEUNG, B. H.-K., LI, L. S., CHIM, A. M.-L., CHAN, C. K.-M., LEUNG, J. K.-Y., CHU, W. C.-W., WOO, J. & CHAN, H. L.-Y. 2018. Beneficial effects of lifestyle intervention in non-obese patients with non-alcoholic fatty liver disease. *Journal of Hepatology,* 69**,** 1349-1356.

WU, T., GAO, X., CHEN, M. & VAN DAM, R. M. 2009. Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: a meta-analysis. *Obes Rev,* 10**,** 313-23.

XIN, Y., DAVIES, A., MCCOMBIE, L., BRIGGS, A., MESSOW, C. M., GRIEVE, E., LESLIE, W. S., TAYLOR, R. & LEAN, M. E. J. 2019. Type 2 diabetes remission: economic evaluation of the DiRECT/Counterweight-Plus weight management programme within a primary care randomized controlled trial. *Diabetic Medicine,* 36**,** 1003-1012.

XUE, Q.-L., WALSTON, J. D., FRIED, L. P. & BEAMER, B. A. 2011. Prediction of risk of falling, physical disability, and frailty by rate of decline in grip strength: the women's health and aging study. *Archives of Internal Medicine,* 171**,** 1119-1121.

YADAV, A., CHANG, Y. H., CARPENTER, S., SILVA, A. C., RAKELA, J., AQEL, B. A., BYRNE, T. J., DOUGLAS, D. D., VARGAS, H. E. & CAREY, E. J. 2015. Relationship between sarcopenia, six-minute walk distance and health-related quality of life in liver transplant candidates. *Clinical transplantation,* 29**,** 134-141.

YANAI, H. 2015a. Nutrition for sarcopenia. *Journal of clinical medicine research,* 7**,** 926.

YANAI, H. 2015b. Nutrition for Sarcopenia. *Journal of clinical medicine research,* 7**,** 926-931.

YILMAZ, Y. & YOUNOSSI, Z. M. 2014. Obesity-Associated Nonalcoholic Fatty Liver Disease. *Clinics in Liver Disease,* 18**,** 19-31.

YKI-JÄRVINEN, H. 2015. Nutritional Modulation of Non-Alcoholic Fatty Liver Disease and Insulin Resistance. *Nutrients,* 7**,** 9127-9138.

YOUNOSSI, Z., ANSTEE, Q. M., MARIETTI, M., HARDY, T., HENRY, L., ESLAM, M., GEORGE, J. & BUGIANESI, E. 2018. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nature reviews Gastroenterology & hepatology,* 15**,** 11.

YOUNOSSI, Z. M., BLISSETT, D., BLISSETT, R., HENRY, L., STEPANOVA, M., YOUNOSSI, Y., RACILA, A., HUNT, S. & BECKERMAN, R. 2016. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology,* 64**,** 1577-1586.

YOUNOSSI, Z. M., RATZIU, V., LOOMBA, R., RINELLA, M., ANSTEE, Q. M., GOODMAN, Z., BEDOSSA, P., GEIER, A., BECKEBAUM, S., NEWSOME, P. N., SHERIDAN, D., SHEIKH, M. Y., TROTTER, J., KNAPPLE, W., LAWITZ, E., ABDELMALEK, M. F., KOWDLEY, K. V., MONTANO-LOZA, A. J., BOURSIER, J., MATHURIN, P., BUGIANESI, E., MAZZELLA, G., OLVEIRA, A., CORTEZ-PINTO, H., GRAUPERA, I., ORR, D., GLUUD, L. L., DUFOUR, J.-F., SHAPIRO, D., CAMPAGNA, J., ZARU, L., MACCONELL, L., SHRINGARPURE, R., HARRISON, S., SANYAL, A. J., ABDELMALEK, M., ABRAMS, G., AGUILAR, H., AHMED, A., AIGNER, E., AITHAL, G., ALA, A., ALAZAWI, W., ALBILLOS, A., ALLISON, M., AL-SHAMMA, S., ANDRADE, R., ANDREONE, P., ANGELICO, M., ANKOMA-SEY, V., ANSTEE, Q., ANTY, R., ARAYA, V., ARENAS RUIZ, J. I., ARKKILA, P., ARORA, M., ASSELAH, T., AU, J., AYONRINDE, O., BAILEY, R. J., BALAKRISHNAN, M., BAMBHA, K., BANSAL, M., BARRITT, S., BATE, J., BEATO, J., BECKEBAUM, S., BEHARI, J., BELLOT, P., BEN ARI, Z., BENNETT, M., BERENGUER, M., BERETTA-PICCOLI, B. T., BERG, T., BONACINI, M., BONET, L., BORG, B., BOURLIERE, M., BOURSIER, J., BOWMAN, W., BRADLEY, D., BRANKOVIC, M., BRAUN, M., BRONOWICKI, J.-P., BRUNO, S., BUGIANESI, E., CAI, C., CALLEJA PANERO, J. L., CAREY, E., CARMIEL, M., CARRIÓN, J. A., CAVE, M., CHAGAS, C., CHAMI, T., CHANG, A., COATES, A., COBBOLD, J., COREY, K., CORLESS, L., CORTEZ-PINTO, H., et al. 2019. Obeticholic acid for the treatment of

non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *The Lancet,* 394**,** 2184-2196.

YUN, K. E., NAM, G. E., LIM, J., PARK, H. S., CHANG, Y., JUNG, H.-S., KIM, C.-W., KO, B.-J., CHUNG, E. C. & SHIN, H. 2016. Waist gain is associated with a higher incidence of nonalcoholic fatty liver disease in Korean adults: a cohort study. *PLoS One,* 11**,** e0158710.

ZELBER-SAGI, S., LOTAN, R., SHLOMAI, A., WEBB, M., HARRARI, G., BUCH, A., KALUSKI, D. N., HALPERN, Z. & OREN, R. 2012. Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. *Journal of hepatology,* 56**,** 1145-1151.

ZELBER-SAGI, S., NITZAN-KALUSKI, D., GOLDSMITH, R., WEBB, M., ZVIBEL, I., GOLDINER, I., BLENDIS, L., HALPERN, Z. & OREN, R. 2008. Role of leisure-time physical activity in nonalcoholic fatty liver disease: a population-based study. *Hepatology,* 48**,** 1791-8.

ZELBER-SAGI, S., SALOMONE, F. & MLYNARSKY, L. 2017. The Mediterranean dietary pattern as the diet of choice for non-alcoholic fatty liver disease: Evidence and plausible mechanisms. *Liver International,* 37**,** 936-949.

ZENITH, L., MEENA, N., RAMADI, A., YAVARI, M., HARVEY, A., CARBONNEAU, M., MA, M., ABRALDES, J. G., PATERSON, I., HAYKOWSKY, M. J. & TANDON, P. 2014. Eight Weeks of Exercise Training Increases Aerobic Capacity and Muscle Mass and Reduces Fatigue in Patients With Cirrhosis. *Clinical Gastroenterology and Hepatology,* 12**,** 1920-1926.e2.

ZIMMET, P., MAGLIANO, D., MATSUZAWA, Y., ALBERTI, G. & SHAW, J. 2005. The metabolic syndrome: a global public health problem and a new definition. *J Atheroscler Thromb,* 12**,** 295-300.

ZWOLINSKY, S., MCKENNA, J., PRINGLE, A., DALY-SMITH, A., ROBERTSON, S. & WHITE, A. 2013. Optimizing lifestyles for men regarded as 'hard-to-reach'through top-flight football/soccer clubs. *Health Education Research,* 28**,** 405-413.

Appendices

Appendix A: Analysis of QOL and WRSM data

Obesity and Weight Loss Quality of Life Measure

The 17 OWLQOL items have a 7-point Likert-like response scale ranging between 0 “Not at all” to 6 “A very great deal”. Before calculating scores, each item is reversed. All OWLQOL items are used with equal weight to derive a single quality of life score. The score is computed by simply summing each item and then transforming this raw score onto a standardized scale of 0 to 100 using the following formula.

Score = (The sum of the component items score (minus) the lowest possible score/ possible raw score range) *100

A score of 0 indicates the greatest impact, and a score of 100 indicates the lowest impact, thus increasing OWLQOL scores imply better quality of life.

Subjects were allowed to miss up to 3 items and still have an analysable score.

Weight-Related Symptom Measure

The WRSM is a 20-item, self-report measure for the presence and bothersomeness of symptoms. A subset of 9 items was specifically targeted to patients with diabetes and thus there is an overall WRSM for all obese patients and a WRSM-D specific for obese patients with diabetes. Participants respond either “yes” or “no” as to whether they have experienced the symptom in the previous 4 weeks and then indicate the degree of bothersomeness that having the symptom caused them. The bothersomeness response options are on a 7-point scale and range from 0 (“not at all”) to 6 (“a very great deal”). A total score is calculated by summing the bothersomeness scores for each symptom. Total scores range from 0 to 120 with higher scores indicating a higher or worse symptom burden.

Appendix B: Patient Information sheet for patients within the studies presented in Chapters 3,4 and 5.

The Liver Unit

Freeman Hospital

High Heaton

Newcastle upon Tyne

NE77DN

Patient Information Sheet

Is a very low calorie diet an acceptable therapy to achieve a target weight loss in patients with advanced non-alcoholic fatty liver disease?

You are invited to participate in this medical research study as a member of your clinical team has screened your medical records and deemed you eligible. Please take time to read the following information carefully. It explains why the research is being done and what it involves. If you have any questions about the given information, you are very welcome to ask for further explanation. Discuss with others if you wish and take time to decide regarding your participation. Thank you for reading this.

What is the purpose of the research project?

Non-alcoholic fatty liver disease (NAFLD) affects up to 1-in-3 adults in Western countries. It represents a spectrum of conditions spanning from simple fatty liver through to non-alcoholic steatohepatitis (NASH – liver inflammation), life threatening cirrhosis, liver cancer and liver failure, and has become a common cause of liver transplant. Risk factors include: increasing age, being overweight or obese, inactivity, Type 2 diabetes mellitus (T2DM) or pre-diabetes, diet and family history. Most patients have no symptoms at diagnosis. Currently, there are no approved drugs to specifically target NAFLD – the main treatment is lifestyle changes with weight loss being the key to improving liver health. Lifestyle interventions designed for weight loss through reduced energy intake in NAFLD have led to reductions in liver fat of between 42 and 81% with the greatest reductions in liver fat observed in people who lose the most weight.

This study aims to assess the feasibility and acceptability of an 8-week very low calorie diet (VLCD) in people with advanced NAFLD. We intend to investigate how many people respond to an invite to take part in this study and how many who take part complete the diet and maintain their weight loss. We are interested in how much weight people lose during the intervention and whether they are able to maintain the weight loss when they return to normal eating in the following six months. We aim to capture peoples' views including what makes it easy or difficult to stick to the diet. This will help us decide whether this type of intervention could be provided on a larger scale to assess its effectiveness, and if effective be used as part of standard clinical care.

This research DOES NOT require a liver biopsy. Previous research has shown that if you lose weight then this improves liver fat and this process is now well understood. Thus we DO NOT feel it necessary to subject you to a "before" and "after" liver biopsy which dramatically reduces the risks involved in taking part in the study.

Who will be suitable for the study?

- People with advanced NAFLD

- People who are aged between 18 and 70 years
- People who are overweight/obese - body mass index (BMI) above 27 kg/m^2
- People who have had a stable body weight for the previous 6 months

Who will not be suitable for the study?

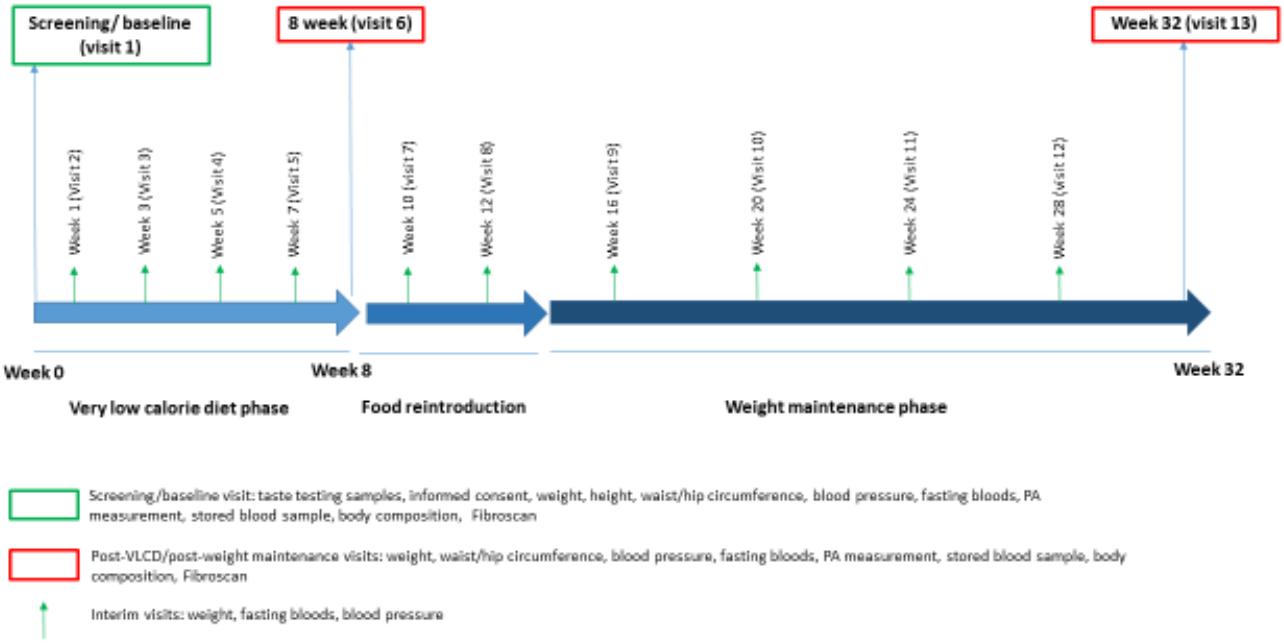
- People with kidney, heart or additional liver problems
- People who are actively losing weight or are currently being treated with anti-obesity drugs
- People who take insulin to control their diabetes
- People with cancer
- People who are on steroids or certain anti-psychotic medications
- People who consume more than the Governments safe alcohol recommendations
- People with highly restrictive dietary preferences
- People who have untreated thyroid disease or active cardiovascular disease
- People with a history of eating disorder
- Anyone who is pregnant/considering pregnancy

Do I have to take part?

Your participation in the study is purely voluntary and does not affect your routine care in any way. You have as long as you wish to decide whether to participate. You will be invited to discuss the study in full with a member of the Research Team. If you decide to take part, you will be asked to sign a consent form only at this stage. If you decide to take part, you can still withdraw at any time without giving reasons and without incurring any bad feelings.

What will the research project involve?

The research involves an 8-week period of very low calorie dieting to induce weight loss, reduce liver fat and improve blood glucose control. You will then be asked to follow a weight maintaining diet and physical activity plan for 6 months with education and support throughout. The diagram below illustrates the basic design of the study.



Weight loss phase (see diagram above and the additional dietary information sheet "<u>The Newcastle Diet – How to do it</u>")

- During the 8 weeks of weight loss a liquid formula diet (Optifast – 600-800 kcal/day) is taken in place of normal meals. In addition you can eat 3 portions of non-starchy vegetables each day and drink at least 2 litres of water or calorie-free beverages each day. You will be asked to abstain from alcohol during the 8-week diet period. The diet will require determination to complete, but will certainly achieve weight loss. During this time, consideration will have to be made with regards to social events, eating out, holidays etc. to allow you to stick to the dietary regimen.

- The Optifast drinks will be provided free of charge for the duration of the study.

- One-to-one support will be provided regularly throughout the diet, both face-to-face at the Freeman Hospital and via telephone or email. You will be asked to weigh yourself at home during the research study and will be given a set of weighing scales to use if you don't already have any. (You will be asked to return these at the end of the study).

- You will be asked to attend the Freeman Hospital on six occasions during the 8-week VLCD phase for monitoring of weight, blood pressure, blood tests and review of how you are getting on with the diet.

- Individualised weight loss targets will be set throughout this time.

Weight maintenance phase (see diagram on previous page)

- During the 6 months of weight maintenance you will be asked to follow either a normal but calorie controlled diet or a Mediterranean style diet. This will be your choice. Meal plans, recipes and snack ideas will be provided. Also, you will be asked either to continue usual activity levels or to become more active.

- You will receive verbal and written information about the diet and activity programmes, some of which are individualised according to your habitual lifestyle.

- You will receive full support from the research team throughout. You will be asked to monitor your weight weekly at home and to attend the Freeman Hospital at monthly intervals for monitoring of weight, blood pressure, blood tests and review of how you are managing the lifestyle changes.

On three occasions during the study period you will be asked to wear a physical activity monitor in the form of an armband for a 7-day period at home.

Main tests

Blood tests will be taken throughout the study and imaging studies will take place on three occasions: before starting the diet, following the VLCD and then after 6 months weight

maintenance. You will be asked to attend the Freeman Hospital having missed out breakfast.

Blood tests will measure your liver enzymes, blood glucose, insulin, lipid profile and markers of inflammation. We will measure your height, weight, hip circumference, waist circumference and blood pressure. At these three visits we will also measure your body composition (the proportion, or percentage, of body fat and fat free mass within the body) and perform a fibroscan to assess your liver stiffness.

We will also select a number of people to undertake a short interview with the Research Team to gather people's experiences of undertaking the study and barriers and facilitators to adopting the very low calorie diet. These interviews will be audio recorded by the Research Team and the anonymised recordings will be sent to "type it write" (a Newcastle based transcription company www.typeitwritetranscription.co.uk) for transcription. The audio recordings are then deleted off the dictaphones and a copy of the recordings and transcriptions will be stored on a password protected folder and only accessed by the Research Team.

What happens after the study?

We will continue to provide you with dietary support and advice after you have completed the study period to enable you to continue with a healthy lifestyle. Telephone advice will continue to be available for at least 3 months. With your permission, we shall also liaise as necessary with your liver doctor and GP.

Expenses and payments

Any travel / parking costs for helping with this research will be refunded. We can arrange a taxi to transport you to and from the Freeman Hospital for your investigations if necessary.

What will I have to do about my current treatments?

If you are taking Sulphonylurea Tablets (Gliclazide, Glimepiride, Tolbutamide) for your diabetes, we will ask you to stop these 3 days before your first study day. If you are taking any other medication for your diabetes, you will be asked to continue these as normal throughout the study unless specifically instructed by a member of the research team. All other medications should be continued as usual. Specifically, if you are on any medication for cholesterol levels, you will be asked to remain on your current dose throughout the study period.

What do I have to do?

We appreciate that this is an intensive study that requires your time and commitment.

- You will be making considerable change in your diet, eating pattern and physical activity levels under specialist supervision and support

- You will be asked to attend the Freeman Hospital on 13 occasions over the 8-month period. This will include blood tests, blood pressure, weight and other investigations. In addition you will be asked how you are getting on with the diet (by a short semi-structured conversation with a member of the research team)

- You will be asked to weigh yourself at home weekly throughout the study period

Is this a new treatment?

No. A very low calorie diet is an established procedure for inducing weight loss which also improves blood sugar control and in some cases can reverse blood sugar problems in people with diabetes.

What are the adverse effects or risks from taking part in this study?

There are no major risks from taking part in this study. The very low calorie diet is safe under medical supervision. The intense dieting and longer-term lifestyle change are challenging but full support will be given.

The low calorie diet can lead to substantial reductions in blood pressure so your medication may be changed to accommodate this.

In some cases, the diet can aggravate gallstones if you have a previous history, but this will be closely monitored.

Some people also report increased constipation during the diet - gentle activity and fluid can help. It should be recognised that emptying the bowels less frequently is normal if you are eating less food. If you suffer any real discomfort we will advise you to see your GP.

If you have a previous history of gout then we will get your GP to check your uric acid levels as they may want to increase your medication if levels rise.

Some people suffer a temporary loss of hair with rapid weight loss. This is transient and hair WILL grow back.

The fibroscan is non-invasive, safe and does not use X-Rays or any other harmful radiation.

Are these any other possible disadvantages of taking part in the research?

You will have to give up time in order to attend the study visits.

What happens at the end of the research project?

We will inform you about the changes in your body weight, blood test results and body composition throughout the study period. We will also by invite you to a volunteer feedback evening at the Freeman Hospital where the results will be explained.

Will my taking part in the project be kept confidential?

All information obtained during the course of the study will be kept strictly confidential. With your permission, your own GP and liver doctor will be informed of your participation in the study.

How will my personal data be used and protected?

The Newcastle upon Tyne Hospitals NHS Foundation Trust (NuTH) is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. NuTH will keep identifiable information about you for 5 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information by contacting Dr Kate Hallsworth (contact details found at the end of this information sheet).

NuTH will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from NuTH and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The Research Team will pass these details to NuTH along with the information collected from you and your medical records. The only people in NuTH who will have access to information that identifies you will be people who need to contact you to arrange appointments as part of the research study or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details. NuTH will keep identifiable information about you from this study for 5 years after the study has finished.

What will happen to the blood samples taken?

The samples will be sent to the laboratories to measure liver enzymes, insulin, glucose, cholesterol and other measurements. Samples will be stored until it is certain that the test results are accurate, and then they will be disposed of. Samples are identified only by code numbers, and not by your name. One sample will be stored after each visit to allow the research team to look at the lipid profile and inflammation in detail. One genetic test will be carried out on the blood samples, to look at the PNPLA3 gene. This gene has been shown to be important in determining liver fat levels. An additional blood sample will be taken at baseline and stored in the UK Biobank so that newer biomarkers for advanced NAFLD can be analysed at a later date if needed. These will be stored for up to 5 years after the study has been completed.

What will happen if abnormal results are discovered during the study?

Any abnormal results from blood tests or other investigations will be explained to you and, with your permission, to your own GP and liver doctor so that appropriate action can be taken if required.

What will happen to the results of the research study?

This study is being undertaken as part of an educational qualification (PhD) and NIHR Clinical Lectureship. The results of the project will be presented in national and international liver meetings and will be published in one of the liver journals. You will not be identified in any report or publication. You will be welcome to have a copy of the results once they are published.

What if relevant new information becomes available during the study period?

This will be relayed to you in writing in a clear manner. If appropriate, your GP will also be informed (with your permission).

What will happen if I don't want to continue with the study?

You will be able to withdraw from the study at any time. Measurements already made can sometimes still be used if you were to agree to this. Withdrawal from the study would not affect your routine care in any way.

What if there is a problem during the course of the study?

If you have any concern or complaint about any aspect of the study, you should contact Dr Kate Hallsworth by telephone on 0191 208 8882, email: kate.hallsworth@ncl.ac.uk or write to her at the address provided below. If you remain unhappy you can contact the Complaints Department in the Trust by telephone on 0191 233 6161, email: patient.relations@nuth.nhs.uk or by letter at Patient Relations Department, The Newcastle upon Tyne Hospitals NHS Foundation Trust, Freeman Hospital, High Heaton, Newcastle upon Tyne, NE7 7DN.

In the unlikely event that something goes wrong and you suffer in any way the arrangements are as follows. If negligence of staff led to harm, then this would be covered by the Newcastle upon Tyne Hospitals NHS Foundation Trust clinical negligence scheme. You may have to meet legal costs.

Who is organising and funding the research project?

This project is funded by a grant from the Wellcome Trust, a PhD Studentship funded by the Newcastle NIHR Biomedical Research Centre and a NIHR/HEE Clinical Lectureship awarded to Dr Kate Hallsworth. The design and organisation of the study is the responsibility of Dr Kate Hallsworth and Dr Stuart McPherson who are recognised as experts in this field.

Who has reviewed the study?

The study has been approved by the North East – Newcastle and North Tyneside 1 Research Ethics Committee.

Who are the contacts for further information?

Further information can be obtained from:

Dr Kate Hallsworth (Senior Research Physiotherapist; NIHR Clinical Lecturer)

Room M4:077

4th Floor William Leech Building

Medical School

Newcastle University

Newcastle upon Tyne

NE2 4HH

Phone: 0191 2088882

Email: Kate.hallsworth@ncl.ac.uk

Dr Stuart McPherson, Consultant Hepatologist

The Liver Unit

Freeman Hospital

High Heaton

Newcastle upon Tyne

NE7 7DN

Phone: 0191 2448753

Email: stuart.mcpherson@nuth.nhs.uk

Thank you very much indeed for considering this study.

Appendix C: Consent form for the study presented in Chapters 3,4 and 5

Is a very low calorie diet an acceptable therapy to achieve a target weight loss in patients with advanced non-alcoholic fatty liver disease?

Volunteer identification number for this study:_____

Consent Form

Please initial each box

1) I confirm that I have read and understood the information sheet for the study (version number ___) and have had the opportunity to ask questions ☐

2) I understand that taking part is voluntary and that I am free to withdraw at any time, without giving reasons, without my medical care or legal rights being affected ☐

3) I agree that I can be contacted at future date and asked to provide information even if I withdraw from the study. ☐

4) I give permission for data extraction from my medical record, about body weight and liver status in the future by the research team, and by authorised representatives of the study Sponsor and relevant regulatory bodies, for the purposes of audit only ☐

5) I agree to provide blood samples which will be tested for PNPLA3 genotype and can be stored for future studies ☐

6) I am happy to be contacted and asked to participate in interviews about my views and experience of the study. ☐

7) I understand that interviews will be audio-recorded and if appropriate, quotes used from the interviews will be anonymised ☐

8) I understand the research team may contact my GP regarding my participation in the study ☐

9) I understand that the investigations are not diagnostic tests but any observation which might be important for health will be explained to me and my GP ☐

10) I understand that my data will be stored securely. ☐

11) I agree to take part in this study ☐

PTO→

Name of participant (please print name).....

Date..... Signature .....

Researcher (please print name).....

Date..... Signature.....

Appendix D: GP letter

RE: Research study participation

Dear Dr XXXX,

We are writing to inform you that XXXXXX from your practice is taking part in our research study titled: **Is a very low calorie diet an acceptable therapy to achieve a target weight loss in patients with advanced non-alcoholic fatty liver disease?**

During this study they will follow an 8-week period of very low calorie dieting to induce weight loss and improve liver health, with regular one-to-one support. This will be achieved through a liquid formula diet taken in place of normal meals. They will then undergo 6 months of weight maintenance, where they will follow either a normal but calorie counted diet or a Mediterranean diet. Following the 8-week intervention, they will make a graduated return to solid food meals. They may be asked to become more active and full support will be received throughout.

Diabetic patients taking Sulphonylurea Tablets (Gliclazide, Glimepiride, Tolbutamide) will be asked to stop these 3 days before the first study day as these medications increase the risk of hypoglycemic episodes with rapid weight loss. Any other diabetic medication should be continued as normal throughout the study unless specifically instructed by a member of the research team. Regular glucose monitoring will be undertaken as part of the study protocol. (Patients taking insulin to control their diabetes will be excluded from the study). All other medications should be continued as usual. Specifically, any medication for high cholesterol levels, will be continued at the current dose throughout the study period. Blood pressure will be monitored regularly as part of the study protocol, and adjustments made to blood pressure lowering medications as required. Any changes to medication will be made by a qualified

member of the research team (Dr S McPherson or a clinical research fellow) and you will be notified straight away.

If you have any questions or require any further information please do not hesitate to contact us on 0191 208 8882 or email: kate.hallsworth@ncl.ac.uk / stuart.mcpherson@nuth.nhs.uk

Kind regards,

Dr Kate Hallsworth

Senior Research Physiotherapist

Dr Stuart McPherson

Consultant Liver Specialist

Appendix E: Semi-structured topic guide used to facilitate 1-2-1 interviews post VLCD intervention

Is a very low calorie diet an acceptable therapy to achieve a target weight loss in patients with advanced non-alcoholic fatty liver disease?

Topic Guide for Patient Interviews (Semi-structured)

These interviews will investigate outcome expectations, general experiences, the challenges participants faced (including, for example, in their social and work environments), and approaches to overcoming them and adhering to the intervention at its different stages.

After 8-week Vlcd

- How did you find the diet? How did it compare to what you expected? What did you expect to get out of the diet?
- Was there anything you thought that might make the diet more difficult to stick to, before you started? (prompt: barriers with work, social events, holidays)
- What if anything made the diet difficult to stick to? (Prompt: any cravings, hunger, temptations, others eating foods you like, social events, holidays)
- How did you overcome these issues?
- What if anything made the diet easy to stick to?
- What advice would you give to others thinking about starting this diet?
- How have you found eating with friends/ family?
- What would make it easier?
- How did you fit this around work/ social events?
- How did you feel about the diet pre-intervention?
- Do you see the VLCD as the start of your weight loss? i.e, with a view to continuing via an alternative method?
- To what extent do you believe this diet had been effective?

Appendix F: Obesity and Weight-loss Quality-of-Life (OWLQOL) instrument used to measure quality of life with the study described in Chapters 3 and 4

Obesity and Weight-Loss Quality-of-Life (OWLQOL) Instrument

© University of Washington, 2004

Your Feelings About Your Weight

Below is a list of statements about your quality of life in relation to being overweight and trying to lose weight. For each of the following statements, please mark an [X] in the one box that best describes your answers at this time.

	Not at all	Hardly	Some-what	Moder-ately	A good deal	A great deal	A very great deal
1. Because of my weight, I try to wear clothes that hide my shape.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
2. I feel frustrated that I have less energy because of my weight.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
3. I feel guilty when I eat because of my weight.	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
4. I am bothered about what other people say about my weight	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

5. Because of my weight, I try to avoid having my photograph taken.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
6. Because of my weight, I have to pay close attention to personal hygiene.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
7. My weight prevents me from doing what I want to do.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
8. I worry about the physical stress that my weight puts on my body.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
9. I feel frustrated that I am not able to eat what others do because of my weight.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
10. I feel depressed because of my weight.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
11. I feel ugly because of my weight.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
12. I worry about the future because of my weight.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐

13. I envy people who are thin.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
14. I feel that people stare at me because of my weight.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
15. I have difficulty accepting my body because of my weight.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
16. I am afraid that I will gain back any weight that I lose.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
17. I get discouraged when I try to lose weight.	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐

Weight-Related Symptom Measure (WRSM)

© University of Washington, 2004

Weight-Related Symptoms and How Much They Bother You

For each of the following questions, read the list of symptoms below, and mark an [X] in the one box that best describes your answer.

a. In the past 4 weeks, did you have the following symptoms?	**b. If Yes, how much did these symptoms bother you?**

No	Yes	SYMPTOMS	Not at all	Hardly	Some-what	Moder-ately	A good deal	A great deal	A very great deal
0☐	1☐	Shortness of breath	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Tiredness	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Sleep problems	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Sensitivity to cold	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Increased thirst	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Increased irritability	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Back pain	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Frequent urination	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Pain in the joints (hips, knees, ect).	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Water retention	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Foot problems	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Sensitivity to heat	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Snoring	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Increased appetite	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0☐	1☐	Leakage of urine	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

0 ☐	1 ☐	Lightheadedness	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0 ☐	1 ☐	Increased sweating	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0 ☐	1 ☐	Loss of sexual desire	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0 ☐	1 ☐	Decreased physical stamina	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
0 ☐	1 ☐	Skin irritation	0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

Please go back to the questions you just answered to make sure you did not miss any items.

Thank you for taking the time to complete the questionnaire

This Obesity and Weight-Loss Quality-of-Life (OWLQOL) Instrument and Weight-Related Symptom Measure (WRSM) was adapted from:

Niero, M., Martin, M., Finger, T., Lucas, R., Mear, I., Wild, D., ... & Patrick, D. L. (2002). A new approach to multicultural item generation in the development of two obesity-specific measures: the Obesity and Weight Loss Quality of Life (OWLQOL) questionnaire and the Weight-Related Symptom Measure (WRSM). *Clinical therapeutics*, *24*(4), 690

Appendix G: Data collected at each study visit

NAFLD VLCD Data

Table of events:

Visit	**1**	2	3	4	5	**6**	7	8	9	10	11	12	**13**
Procedure													
Height	X												
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X
Waist circumference	X	X	X	X	X	X	X	X	X	X	X	X	X
Hip circumference	X	X	X	X	X	X	X	X	X	X	X	X	X
PA/Sleep	X					X							X
Blood pressure	X	X	X	X	X	X	X	X	X	X	X	X	X
Body composition	X					X							X
Fasting glucose	X	X	X	X	X	X	X	X	X	X	X	X	X
Bloods:													
Lipid profile	X	X	X	X	X	X	X	X	X	X	X	X	X
LFTs (+AST+GGT)	X	X	X	X	X	X	X	X	X	X	X	X	X
Hba1c	X					X							X
Insulin	X					X							X
Full blood count	X	X	X	X	X	X	X	X	X	X	X	X	X
Stored sample for lipidomics/inflammatory markers	X	X	X	X	X	X	X	X	X	X	X	X	X
Genotyping	X												
FIB-4/NFS	X					X							X
QRisk2	X					X							X

Record of any side effects/AEs	X	X	X	X	X	X	X	X	X	X	X	X	X
Fibroscan	?*					X							X
Weight-related QoL Q	X					X							X
Semi-structured interview						X							X

*if most recent scan >3months ago

Appendix H: Newcastle Diet Plan

The Newcastle Diet – How to do it

The Newcastle diet (Lean et al.) is an eight-week very low calorie diet (VLCD, less than 800 calories per day) that has been shown to reduce liver fat in many people. It is important to bear in mind the diet is only eight weeks long. It is followed by a return to normal eating but with reduced calorie intake than previously. You will not be missing out on the things you enjoy for too long!

What will I eat and drink?

During the 8-week weight-loss phase you will have the following each day:

- ☑ Meal replacement products (Optifast soups or shakes, 3 sachets – these will be provided free of charge for the 8-week weight-loss phase) – this provides a total of 600 calories. You must have all 3 sachets to ensure you get adequate protein (vital to maintain muscle mass during weight loss) and essential vitamins and minerals. Make up according to the instructions on the packet*.
- ☑ Eat 3 portions of vegetables (not fruit), for fibre content and additional nutrients. This will provide up to 200 calories.
- ☑ Drink 2 litres (4 pints) of water or calorie-free beverages each day *in addition* to the fluids from the meal replacement products.
- ☑ You can use up to 100ml allowance of skimmed or semi-skimmed milk for tea and coffee throughout the day (optional).
- ☑ No alcohol while following a VLCD (it is very high in calories)

* Nothing else is eaten during the 8-week weight loss phase. The above meal plan replaces all of your usual foods (meals, snacks and calorie containing drinks). The only exception to this is milk where this is a requirement for making up your meal

replacement products. Follow the instructions on the packet as these vary by product used. Do not substitute water if the instructions direct you to use skimmed or semi-skimmed milk, as you will miss out on vital protein and nutrients.

Why does the diet use liquid meal replacements and not 'real' food?

It isn't essential to follow a VLCD to achieve results. Many people have followed food-based diets and lost weight gradually over a longer period of time. The key is taking in fewer calories than you burn. This allows enough weight loss to strip the fat out of the liver and pancreas. We have chosen meal replacements for the Newcastle diet as they have the following advantages:

- They are filling and hunger is not a major problem for most people
- They are simple to use provide a break from making decisions about what and how much to eat
- The complete change gives an opportunity to break unhelpful eating habits
- They provide complete nutrition in a small amount of calories
- They are quick and easy to prepare
- They allow rapid weight loss – you see and feel the results quickly. We know that losing weight more quickly does not cause faster weight regain than losing weight more slowly. In fact, the more weight people lose in the first 6 months of a diet the more weight they tend to keep off over the longer term
- Times when you are tempted to eat or snack stand out more, helping you work out your triggers for eating and to plan more helpful ways to manage them
- At the end of the 8 weeks you have a 'blank slate' to start your new eating habits for life

What can I expect?

Your body will rapidly begin to use up fat stores as its energy source. It may take a few days to adapt. During this period you may experience some side effects, largely due to fluid shifts which occur.

Possible side effects include:

During the first few days	Throughout the 8 weeks
Headache: Ensure fluid requirements are met; use over the counter painkillers if required	*Hunger:* If it persists try moving the timing of your meal replacements and vegetables to avoid long gaps. A drink of sparkling water also helps to fill you up
Dizziness: Ensure fluid requirements are met; take your time when standing up or changing position; see your GP for review if taking medication to lower blood pressure	*Constipation:* Largely prevenTable with adequate fluid, fibre and movement. Ensure you are eating your vegetable allowance each day; drink adequate fluid; undertaking some gentle activity; see your GP if constipation persists.
Tiredness: Plan to start the diet when you don't have any strenuous activities planned for the first few days. Weight loss will come from the cut in calories so you can take it easy until you are used to the diet and your energy levels increase (usually after the first couple of days).	*Increased sensitivity to cold:* A result of reduced body fat which provides extra insulation. Wrap up warm, have warming drinks such as tea/coffee, try meal replacement soups instead of shakes.
Hunger: This usually wears off after the first few days. Keep busy to take your mind off food – go for a walk or do some jobs around the house that you keep putting off.	*Hair loss:* Some people may experience hair loss/thinning, usually around 2-3 months after starting the VLCD. This is a result of more hairs being in the 'falling out' stage (rather than the growing stage) at the same time. It is temporary and your hair will grow back normally in time.

Many of the above can be helped by having some additional salt (equivalent to a level teaspoon) in your first few days. Add a little salt or soy sauce to your vegetables or try a drink of Bovril or vegetable bouillon (stock) twice a day.

Vegetables

You should eat a total of 3 portions of vegetables every day. At least 1x portion of green leafy vegetables plus 2x portions of other vegetables. Below is a list of vegetables you can eat: Try them steamed, grilled, dry fried, stir fried or wrapped in tin foil and roasted in the oven. Try to avoid boiling as nutrients will be lost in the cooking water.

Green Leafy Vegetables (1x 80g portion):

Cabbage	Spinach	Kale	Broccoli
Pak Choi	Kohlrabi	Swiss Chard	Collard greens

Other vegetables (Max 2x 80g portions):

Onions/shallots	Bean Sprouts	Carrots	Lettuce	Leeks	Mushrooms
Peppers	Artichoke	Radish	Water Chestnuts	Brussel sprouts	Turnip/Swede
Aubergine	Spring onion	Celeriac	Fresh/tin tomatoes	Courgette	Peas
Okra	Sugar-snap peas	Cauliflower	Asparagus	Green beans	Fennel

- **Avoid:** potato, sweet potato, parsnip, sweet corn squash, yam, avocado, olives, nuts, seeds,

  pulses, fruit, coleslaw

Herbs, Spices and Flavourings

Eating vegetables need not be boring and so the following Tables give examples of how to add flavour to your veg as well as recipe ideas to get you started.

Basil	Lemon/lime juice	Parsley	Oregano	Balsamic vinegar
Tarragon	Chilli powder	Cinnamon	Rosemary	Curry powder
Thyme	Dried chillies	Fresh Chillies	Coriander	Ginger
Turmeric	Garlic	Cumin	Black pepper	Sage
Harissa paste	Soy sauce	Malt vinegar	Piri Piri seasoning	Chinese 5 spice

1tsp vegetable or rapeseed oil	1tbsp light salad dressing	5g butter/ margarine	1tsp coconut oil	1tsp Olive oil

<u>Dressings, Fats and Oils (Max 1 portion/day)</u>

Dressings and oils are high in calories and so either low calorie or only small amounts should be used during the diet. A small amount of fat adds flavour however and helps absorption of fat soluble vitamins.

Return to normal eating

Once you have completed the 8-week VLCD you are ready for the next stage, where you will return to eating normal foodstuffs. It can be difficult to know what to eat and how much, especially if you have lost and regained weight in the past. We advise taking things one step at a time, reintroducing meals over a 2-week period as you gradually cut down on the diet replacement products (soups and shakes). This is the perfect time to improve the quality and variety of your diet by trying new foods

Step 1:

Snack Ideas

- Sugar snap peas/mange tout
- Kale crisps
  - Mix 2 handfuls kale with 1tsp oil, pinch smoked paprika, salt and pepper
  - Spread on a baking tray and bake at 150^{o}C for 15minutes.
  - Sprinkle with lemon juice and zest
- Handful cherry tomatoes
- Cubes raw turnip
- Carrot and celery sticks with homemade salsa
  - For the salsa, finely chop 4 fresh plum tomatoes, 1 bunch of rocket and 1 bunch of flat leafed parsley and mix together
  - Chop carrots and celery into 4cm sticks and dip in the salsa!

Recipe Ideas

Pea and spinach soup (3 veg portions)	
Ingredients • 1 tsp olive oil • ½ onion, finely chopped • 1 garlic clove, chopped • 80g fresh peas, podded • 80g baby spinach • 300ml hot vegetable stock • salt and freshly ground black pepper • 1 tbsp chopped fresh chives	Method 1. Heat the olive oil in a saucepan, add the onion and fry gently until softened but not coloured. Add the garlic and fry for one minute. 2. Add the peas, spinach and hot stock and bring to the boil. Reduce the heat and simmer for eight minutes, or until the peas are tender. Season with salt and freshly ground black pepper and blend with a hand-blender until smooth. 3. To serve, pour into a bowl and garnish with chopped fresh chives and a drizzle of olive oil.

Aubergine salad (serves 2; 3 veg portions per serving)

Ingredients	Method
• 1 medium aubergine (240g) • 40g spring onions • 40g cherry tomatoes, cut into quarters • 80g small red pepper, deseeded and finely diced • 80g small green pepper, deseeded and finely diced • 1 tbsp chopped fresh mint Dressing: • juice ½ lemon • ½ small red chilli (deseeded if you don't like it too hot), finely chopped (optional) • 1 small garlic clove, crushed • 1 tbsp extra virgin olive oil	1. Heat oven to 200C/180C fan/gas 6 and line a baking tray with foil. Prick the aubergine with a sharp knife to prevent it from exploding, then put it on the prepared tray and roast for 45-55 mins until the skin is wrinkled and it is very soft. 2. While the aubergine is roasting, make the dressing. Mix together the lemon juice, chilli, if using, garlic and olive oil in a bowl. Season and set aside. 3. When the aubergine is cool enough to handle, peel and place it in a colander. Press down on it very gently over a bowl to allow the juices to run out, then transfer the aubergine to a serving plate and cut into large pieces. Dress quickly with half of the dressing, then add the spring onions, cherry tomatoes and peppers to the plate. Pour over the remaining dressing and mix with your hands or a spoon to coat. Serve warm, scattered with mint.

Indian spiced greens (serves 2; 2 veg portions per serving)	
Ingredients • 2 tsp vegetable oil • ½ tsp cumin seed • ½ tsp mustard seed • 0-2 green chillies (depending on taste) finely chopped • large piece fresh root ginger • ½ tsp turmeric • 240g shredded greens, such as kale, Brussel sprouts, or any other • 80g peas • Juice 1/2 lemon • ½ tsp ground coriander • small bunch coriander, roughly chopped	Method 1. Heat the oil in a large non-stick pan or wok, sizzle the cumin and mustard seeds for 1 min, then add the chilli, ginger and turmeric. Fry until aromatic, then add the greens, a pinch of salt, a splash of water and the peas. 2. Cover the pan and cook for 4-5 mins until the greens have wilted. Add the lemon juice, ground coriander and half the fresh coriander then toss everything together. Pile into a serving dish and scatter with the rest of the coriander.

Cauliflower rice (80g = 1 veg portion)	
Ingredients • 1 medium cauliflower • good handful coriander, chopped • cumin seeds, toasted (optional)	Method 1. Cut the hard core and stalks from the cauliflower and pulse the rest in a food processor to make grains the size of rice. Tip into a heatproof bowl, cover with cling film, then pierce and microwave for 7 mins on High – there is no need to add any water. Stir in the coriander. For spicier rice, add some toasted cumin seeds.

Vegetable curry (serves 4; 2 veg portions per serving)	
Ingredients • 1 tbsp vegetable oil • 1 large onion, thickly sliced • 1 large garlic clove, crushed • 2 tbsp curry powder • 2 large carrots, thickly sliced • 200g turnip, cut into chunks • 400g can chopped tomato • 425ml hot vegetable stock • 4 tbsp chopped coriander, plus extra to serve	Method 1. Heat the oil in a large pan, then add the onion and garlic and cook gently, stirring occasionally, until the onion softens, about 5-8 minutes. Stir in the curry powder. 2. Tip the fresh vegetables into the pan and add the tomatoes and stock. Stir in 3 tbsp of the coriander. Bring to the boil, turn the heat to low, put the lid on and cook for half an hour. 3. Remove the lid and cook for another 20 minutes until the vegetables are soft and the liquid has reduced a little. There should be some liquid remaining, but not too much. Season with salt and pepper. 4. Ladle the curry onto cauliflower rice

Appendix I: Nutritional breakdown of Optifast meal replacement products

Typical values	Per 100g	Per 54g in 200ml of water
General		
Energy kJ/kcal	1620/385	875/208
Protein (34% kcal) g	32.4	17.5
Carbohydrates (43% kcal) g	41.7	22.5
of which sugars g	34.2	18.5
Fat (19.5% kcal) g	8.3	4.5
of which saturates g	2.9	1.6
of which monounsaturates g	2.9	1.6
of which polyunsaturates g	2.5	1.35
linoleic acid g	2.1	1.13
Omega-3 g	0.4	0.21
Fibre (3.5% kcal) g	6.7	3.6
Vitamins		
A µg RE	830	448
D µg	4.4	2.4
E mg α-TE	20.8	11
K µg	55	30
C mg	80	43
B1 (Thiamin) mg	1.2	0.65
B2 (Riboflavin) mg	1.6	0.86
B6 mg	1.4	0.76
Niacin mg NE	24	13
Folic acid µg	230	124
B12 µg	2.6	1.4
Pantothenic acid mg	6	3.2
Biotin µg	25	13.5

Minerals		
Sodium g/mmol	0.36/15.6	0.19/8.3
Chloride mg/mmol	470/13.2	254/7.1
Potassium mg/mmol	1500/38.4	810/20.7
Calcium mg/mmol	650/16.3	351/8.8
Phosphorus mg/mmol	620/20	335/10.8
Magnesium mg/mmol	230/9.5	124/5.1
Iron mg	11	5.9
Zinc mg	10.6	5.7
Copper µg	1	0.54
Iodine µg	180	97
Selenium µg	60	32.4
Manganese mg	1	0.54
Chromium µg	110	59.4
Molybdenum µg	80	43.2
Fluoride mg	560	302
Osmolality mOsm/kg	600	

Appendix J: Protocols for management of inadequate blood glucose control and blood pressure

Adapted from the DiRECT protocol:

Leslie, W.S., Ford, I., Sattar, N. *et al.* The Diabetes Remission Clinical Trial (DiRECT): protocol for a cluster randomised trial. *BMC Fam Pract* **17,** 20 (2016). https://doi.org/10.1186/s12875-016-0406-2

Antihyperensive drugs

1. In the first 2 weeks after stopping antihypertensive and diuretic medication:

   If systolic BP >165 mmHg on repeated measurement - restart one drug, as below.

2. Thereafter, if systolic BP is >140 mmHg - restart one drug as below.

3. Increase dose weekly to achieve target.
4. If systolic BP remains >140 mmHg on the first drug - add a second drug, as below.
5. Increase dose weekly to achieve target.
6. Repeat as necessary with third, fourth or more drugs (increasing each to maximum dose).

Order of reintroduction (previously used drugs)

1. ACE inhibitors (ramipril. lisinopril, perindropril, etc.)

2. Angiotensin receptor blockers (irbesartan, candesartan etc.)

3. Thiazide (bendroflumethazide, indapamide etc.)

4. Spironolactone

5. Calcium channel blocker (nifedipine, amlodipine etc.)

6. Beta blocker (atenolol, labetolol etc.)

7. Alpha blocker (doxazosin, prazosin)

8. All others

Glucose control

1. After 2 weeks of VLCD if osmotic symptoms (thirst, polyuria) are troublesome or if random capillary glucose is over 20 mmol, check HBA1c and that weight loss is as anticipated.
2. If it is not, discuss whether any other help would be helpful with following the low calorie liquid diet.
3. If weight loss is satisfactory but control is still inadequate, consider introducing an oral hypoglycaemic agent.
4. Start at the lowest dose and increase gradually.
5. Subsequently, if control remains poor, add further agents
6. Urge further weight loss at each visit

Order for reintroduction of anti-diabetic medications

1. Reintroduce metformin (500 mg bd). If this has previously caused GI upset for the individual, use the slow release preparation.
2. Increase metformin to a usual maximum of 1 g BD over 2–4 weeks
3. If a second agent is required, add sitagliptin 100 mg od.
4. If, after 4 weeks, control is still inadequate, add gliclazide 80 mg od (or other sulphonylurea if previously used, or if preferred).
5. Increase sulphonylurea dose gradually until glucose control is adequate
6. Use current diabetes guidelines if glucose control remains inadequate

Appendix K: COREQ checklist for reporting qualitative data

COREQ (COnsolidated criteria for REporting Qualitative research) Checklist

A checklist of items that should be included in reports of qualitative research. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Topic	Item No.	Guide Questions/Description	Reported on Page No.
Domain 1: Research team and reflexivity			
Personal characteristics			
Interviewer/facilitator	1	Which author/s conducted the interview or focus group?	
Credentials	2	What were the researcher's credentials? E.g. PhD, MD	
Occupation	3	What was their occupation at the time of the study?	
Gender	4	Was the researcher male or female?	
Experience and training	5	What experience or training did the researcher have?	
Relationship with participants			
Relationship established	6	Was a relationship established prior to study commencement?	
Participant knowledge of the interviewer	7	What did the participants know about the researcher? e.g. personal goals, reasons for doing the research	

Interviewer characteristics	8	What characteristics were reported about the inter viewer/facilitator? e.g. Bias, assumptions, reasons and interests in the research topic	
Domain 2: Study design			
Theoretical framework			
Methodological orientation and Theory	9	What methodological orientation was stated to underpin the study? e.g. grounded theory, discourse analysis, ethnography, phenomenology, content analysis	
Participant selection			
Sampling	10	How were participants selected? e.g. purposive, convenience, consecutive, snowball	
Method of approach	11	How were participants approached? e.g. face-to-face, telephone, mail, email	
Sample size	12	How many participants were in the study?	
Non-participation	13	How many people refused to participate or dropped out? Reasons?	
Setting			
Setting of data collection	14	Where was the data collected? e.g. home, clinic, workplace	
Presence of nonparticipants	15	Was anyone else present besides the participants and researchers?	
Description of sample	16	What are the important characteristics of the sample? e.g. demographic data, date	
Data collection			
Interview guide	17	Were questions, prompts, guides provided by the authors? Was it pilot tested?	
Repeat interviews	18	Were repeat inter views carried out? If yes, how many?	

Audio/visual recording	19	Did the research use audio or visual recording to collect the data?	
Field notes	20	Were field notes made during and/or after the inter view or focus group?	
Duration	21	What was the duration of the inter views or focus group?	
Data saturation	22	Was data saturation discussed?	
Transcripts returned	23	Were transcripts returned to participants for comment and/or	
Topic	**Item No.**	**Guide Questions/Description**	**Reported on Page No.**
		correction?	
Domain 3: analysis and findings			
Data analysis			
Number of data coders	24	How many data coders coded the data?	
Description of the coding tree	25	Did authors provide a description of the coding tree?	
Derivation of themes	26	Were themes identified in advance or derived from the data?	
Software	27	What software, if applicable, was used to manage the data?	
Participant checking	28	Did participants provide feedback on the findings?	
Reporting			
Quotations presented	29	Were participant quotations presented to illustrate the themes/findings? Was each quotation identified? e.g. participant number	

Data and findings consistent	30	Was there consistency between the data presented and the findings?	
Clarity of major themes	31	Were major themes clearly presented in the findings?	
Clarity of minor themes	32	Is there a description of diverse cases or discussion of minor themes?	

Developed from: Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. *International Journal for Quality in Health Care*. 2007. Volume 19, Number 6: pp. 349 – 357

Once you have completed this checklist, please save a copy and upload it as part of your submission. DO NOT include this checklist as part of the main manuscript document. It must be uploaded as a separate file.

Appendix L: UKELD and MELD normative values

Current and proposed mathematical models for organ allocation (Asrani and Kim, 2010)

Model	Equation	Notes
MELD	$9.57 \times \log_e(\text{creatinine, mg/dl}) + 3.78 \times \log_e(\text{total bilirubin, mg/dl}) + 11.2 \times \log_e(\text{INR}) + 6.43$	Lower limits of the individual components are bound by 1 and creatinine is capped at 4 mg/dl
UKELD	$5 \times [1.5 \times \log_e(\text{INR}) + 0.3 \times \log_e(\text{creatinine, µmol/l}) + 0.6 \times \log_e(\text{bilirubin, µmol/l}) - 13 \times \log_e(\text{serum sodium, mmol/l}) + 70]$	Minimal listing criteria: projected 1 year liver disease mortality without transplantation of >9% (UKELD ≥49)

INR, international normalized ratio; MELD, model for end-stage liver disease; UKELD, United Kingdom MELD.

Appendix M: Topic guide used to guide focus group

Focus Group Questions

1. Could I start by asking if anyone has ever taken part in a home based exercise/ physical activity programme before? (How did you find it?)- Was there anything you liked or disliked about the programme?

2. Why did you chose a home-based programme? (What did you feel motivated you?)

3. If you were asked to complete an exercise programme at home, what sort of information would you need to help you decide whether or not to do? (Prompt- benefits, time it will take to do, an idea of what it would consist of, goals/ targets)

4. What are your thoughts about having a number of tests taken before and after an exercise programme? These might include weight measurement, questionnaires asking about your health and quality of life, waist measurement, grip strength test, CPEX)

5. What sort of extra support might be needed to complete a home based exercise programme? (Prompt – equipment, support from another person who/ where from?)

6. What sort of equipment might be useful to help you to complete an exercise programme at home?

7. What might be the main obstacles to completing a home based exercise programme? (Prompt- other health issues, time, costs, emotional or practical support)

8. How do you feel about using technology to access or to provide information? (Prompt- online diaries to record exercise, step counters and to see up to take videos or tips for exercises)

9. What might be the issues with completing an exercise programme outside of the hospital environment – if any? (Prompt: fear of exercising)

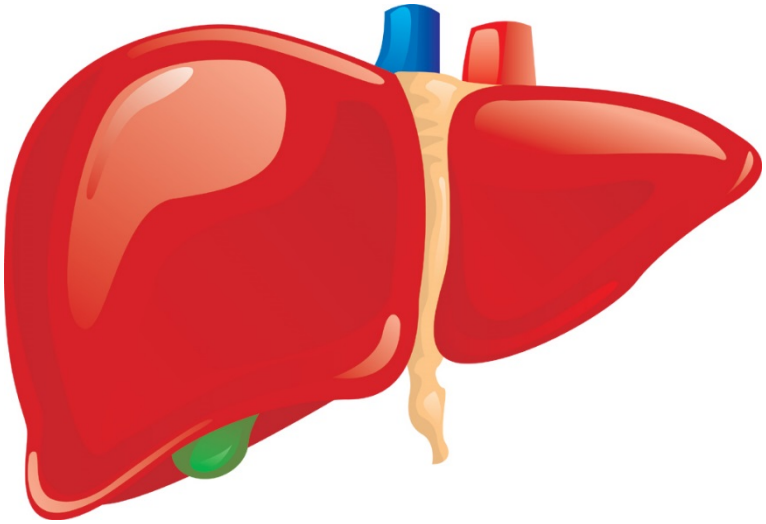
10. What would you hope to achieve from an exercise programme?

11. What would be the best way for you to learn how to complete the exercises within the programme properly? (Prompt- shown in person, paper diagrams, online videos)

Appendix N: Patient facing materials for the developed exercise programme described in Chapter 6

Keeping active on the liver transplant waiting list; your personal exercise plan

Participant Information Guide



What is the purpose of this programme?

Modern research has shown that patients who remain physically active and fit before their liver transplant are less likely to suffer any complications during or after the transplant, and are more likely to make a quicker recovery. Being in the fittest shape possible could help your new liver work at it's best following your transplant. Patients who need a liver transplant often have a higher risk of developing Type 2 Diabetes, heart disease and certain types of cancer, and keeping fit, active and strong can help reduce this risk.

This booklet is your personal, individualised exercise and activity plan, which you have had a role in creating. This booklet can also be used to help you monitor your progress. It has also been developed to keep you informed about the importance of this intervention and has some important information and contact details.

Following the start of your programme, if you have any questions, or would like more information about your personalised exercise plan, please use the contact details below. If you feel like you struggle with your personal plan, or would like to increase the intensity, please use the following contact information;

Email: j.h.scragg@ncl.ac.uk

Telephone: 0191 2088264

If you start to feel unwell during/ following exercise at any time, please stop immediately and contact your healthcare team.

Beginning my programme

The time spent on the waiting list for a liver transplant can vary a lot between individuals. The longer you can spend on improving your fitness by increasing your levels of activity and exercise, the better, and the bigger the difference you'll see following your transplant. For example- you may be able to leave hospital sooner and may start feeling healthier and being more active sooner after your transplant. Therefore, it is recommended to start increasing your activity levels as soon as possible.

There are lots of reasons why patients may need a liver transplant, and therefore patients on the waiting list for a liver transplant will most likely vary a lot in how well or unwell they feel. Some patients may be referred onwards for an individualised exercise or nutrition plan. If you already exercise regularly or are very active, it is good to try and keep this up for as long as possible. Following your operation, you will need a period of time to recover and you should discuss returning to exercise with your doctor prior to restarting your exercise.

If you currently do not exercise regularly, it is advised to start with small amounts of activity and low intensity exercises, and then gradually build this up to a manageable level over the duration of your time on the waiting list. It is common that patients may start to feel worse in the build up to their transplant and be less able to exercise to a high intensity, and therefore is important to not overwork yourself, and keep exercising at a manageable level. Similarly, if you start to feel more confused or unstable, it is important to adapt your exercise to minimise the risk to yourself. For example, this may mean exercising with a companion, or partaking in mainly floor based exercises.

People with liver disease can have days when their symptoms are worse and it is more difficult to stay active. For example, ascites (fluid build up around the abdomen) and hepatic encephalopathy (confusion) are common. Try and stay positive on these days, and try to keep as active as possible. Over an extended period of time, if you are struggling maintain your activity levels, please contact us using the available contact details.

Getting started

Your individualised exercise plan should include a combination of activities to improve your cardiorespiratory system, by strengthening your heart and lungs, muscular exercises to help build up the strength in your muscles and exercises designed to help you improve your stability.

If you have any pre-existing muscular, joint or skeletal problems, then exercises that affect those areas should be adapted or missed out entirely.

During the programme, on the scale provided below, you should aim to be working at level of 3 when doing your exercise.

Score	How should this feel?
1	Resting
2	Easy
3	Getting harder but still comfortable
4	Hard
5	Unable to work any harder

Additionally, if you wish to monitor your activity using an alternative method, then you can use the talk test.

Talk Test

The **talk test** is a simple way to measure how hard you're working. In general, if you're doing moderate-intensity activity **you can talk, but not sing**, during the activity. If you're doing vigorous-intensity activity, you will not be able to say more than a few words without pausing for a breath.

If you want to do your exercises outside of your home/ in the community, you can take this booklet and show staff. This will give them a greater idea of what your fitness levels are currently like and what exercises you have been doing.

Your plan

Following your group training session, you should have an idea about what your exercise plan will look like. Take a moment to write down your personalised plan below:

Cardiorespiratory:

Muscular:

Stability:

At the end of the leaflet, there are tables where you can document how much exercise you have done, and how hard you worked when completing them. Each exercise comes with a code, for example, C1. To make documenting your exercise easier, you can use these codes in the table. In the table, there is also a score section- this is a score of how hard you worked doing that exercise, as mentioned above.

Improving your cardiorespiratory fitness

It is important to improve your cardiorespiratory fitness (how efficiently your heart and lungs work, and how efficiently your body uses oxygen) because people who need a liver transplant could have a higher risk of developing heart disease or having a heart attack. Improving your cardiorespiratory fitness therefore can reduce the risk of heart or lung disease related complications before and after your transplant.

You can make small changes in your lifestyle to increase the amount of cardiovascular activity you do, for example;

- Get off the bus or out a taxi a little earlier and walk part of the way to you destination
- Walk instead of driving
- Park further away from your destination
- Try and walk for a bit longer when shopping

- Use stairs instead of a lift, for at least one or two floors
- If you have a dog, walk it for a little longer than normal
- Increase your activity around the house, by increasing the amount of housework you do

It is important to make small changes and set small targets in regards to increasing your activity. Ensure that your target is realistic and achievable.

There are a variety of exercises that benefit your heart and lungs, including walking, jogging, cycling (using an exercise bike if need be), swimming or climbing stairs. Any activity that raises your heart rate or gets you a little breathless could be considered a cardiorespiratory exercise.

Your cardiorespiratory exercises:

When partaking in cardiorespiratory exercises, ensure that you exercise to a point when you are breathless but can still hold a conversation. If you don't feel safe or comfortable exercising alone, always exercise with a friend or family member. If you don't feel well balanced, your exercises will be predominately floor based, or modified to work within your comfort zone.

Your plan: pick one of the following to do every day, for 10 minutes:

C1. Targeted walking times or step goals

If you have a pedometer, or have a smart phone that counts your steps, this is great way to measure your activity throughout the day. However, if you aim to spend a set amount of time walking, this is also a good way to incorporating cardiorespiratory fitness into your day. If you don't know your staring fitness in terms of walking, start by setting aside ten minutes and seeing for how long you can continuously walk around the house, and monitor how often you need to take a break. If you need to take breaks, one of the first targets will be looking at reducing

breaks/ time spent taking breaks. If you don't find this challenging, you can increase the time spent walking, and then look at increasing the intensity/ speed that you walk.

C2. Stair climbing or step-ups

If you have stairs at home, and feel comfortable/ stable when using them, you can use your stairs to exercise. You can do this by repeatedly climbing the flight of stairs, or doing step ups. This is a high intensity exercise so it may be more feasible to count how many stairs you can climb without stopping using the talk test to identify how hard you're working, or count how many step-ups you can do (nb, count step ups by counting how many steps you climb up, don't include the steps that you climb down).

C3. Targeted swim distance/ swim time

Swimming will provide a low intensity, gentle exercise. This might be particularly good for people with joint or back problems. Start gently and try to build the time or distance/ laps that you can swim. For some people, even just walking around the pool will be challenging enough.

C4. Exercise bike

Aim to start pedalling at a low resistance at a low speed for the first few days to give yourself an understanding of what your body is capable of, and how it feels to do it on sequential days. You can then gradually work your way up to find a level that puts in the right place on the talk test. When progressing, initially aim to increase the time spent cycling, or the speed that you cycle, and then then increase the resistance.

Improving your muscular strength:

Patients with liver disease often lose a lot of muscle definition and muscular strength. Alongside cardiorespiratory exercises, to strengthen the heart and lungs, it is important to strengthen our skeletal muscles. These include our arms, legs and core stomach muscles. Depending on the number of repetitions (reps) you do, and on your current level of fitness, you may find that you start working your cardiorespiratory system. This is normal, but just remember to not work harder than 'hard but comfortable'. You can also use the talk test in these exercises.

Important things to remember:

- As with cardiovascular activity, it is important to set yourself targets and discover what your current ability is. To build up strength, we will increase the reps that you do, or increase the weight of some household items which we can use to build strength.
- If you have any pre-existing conditions (including those that might affect your arms, legs, back or hip) that might affect your exercises, let us know and we may be able to modify exercises to suit your needs or you may be referred to a physiotherapist for a modified programme.
- You may find some exercises harder than others. This may be because they're working different muscles- this is normal and do not worry. Try and work within your comfortable limits.
- If any of the exercises make you feel unwell in any way, please stop and rest. When you feel rested, try to move onto the next exercise, but if you still feel unwell after 10 minutes, then stop your exercises for the day.
- Try and keep each movement controlled, and try not to rely on momentum too much.
- Some exercises ask you to hold a position for a period of time- try to not count too quickly, and if you can't hold for the targeted length of time, that is fine, rest and aim to build up to your target.

Your plan: pick **5** of the following to do, **2-3** days per week (in addition to your cardio exercises).

Your muscular strength exercises:

- **Bicep curls- one bicep at a time (M1)**

  Stand with your feet apart at about shoulder width apart, or sit in an upright and comfortable position. Hold your arms out in front of you, parallel to the floor with palms facing upwards, and slowly bend one arm until your hand reaches your shoulder. Repeat this with the other hand.
  Start with 5 repetitions for each arm.

  Progress this by holding increasing weight- water bottles with measured amount of liquid can help document progress, for example, empty water bottle- 100ml- 300ml- 600ml- 1L. You can also use other household items, such as a can of beans or bags of sugar.

- **Forward shoulder raises- one arm at a time (M2)**

  Stand with your feet shoulder width apart, or sit comfortably in an upright position on a chair. Start with your arms relaxed and by your sides. Slowly raise your arm (straight) and hold it out in front of you at shoulder level. Hold for a count of 5 and slowly lower it to its starting position. Repeat with the other arm.

  Progress this by holding for longer, or holding a small weight in your hand while you do this, such as those mentioned above.

- **Sideways shoulder raises- one arm at a time (M3)**

  Stand with your feet shoulder width apart, or sit comfortably in an upright position on a chair. Start with your arms relaxed and by your sides. Slowly raise your arm (straight) and hold it outwards at shoulder level. Hold for a count of 5 and slowly lower it to it's starting position. Repeat with the other arm.

  Progress this by holding for longer, or holding a small weight in your hand while you do this, such as those mentioned above.

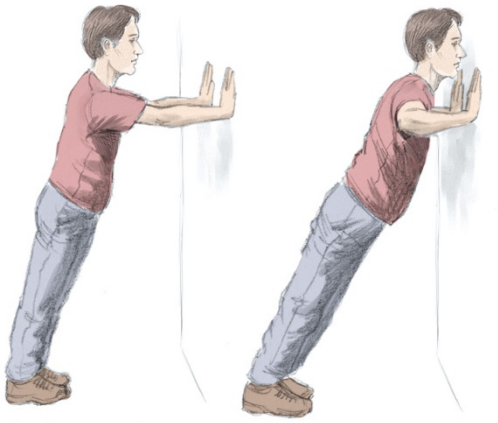
- **Overhead arms (M4)**

  Sit or stand with arms on chest. Raise one arm straight above your head. Hold for 5, and slowly lower your arm back to your chest. Repeat with other arm.

  Progress this by increasing time that arms can be held overhead, or can be progressed to doing this with weights such as water bottles, as above.

- **Wall press ups (M5)**

  Stand facing a wall with your hands at shoulder height, touching wall. Start with your feet shoulder width apart. With your arms pressed against the wall, slowly bend your arms like a press up to bring your body closer to the wall, like a press up. Aim to do 10 to start with.



  Progress this increasing the number of press ups you do.

- **Assisted squats (M6)**

  Stand upright behind a chair, or other supportive furniture. Hold onto the chair with both hands for support. Slowly bend your knees and keep your back straight and heels on the floor. Hold this for a count of 5 and then push your legs straight to come back into a stand position. Aim to do 5 reps.

  Progress this by holding for longer count or increasing repetitions.

- **Squat against wall (M7)**

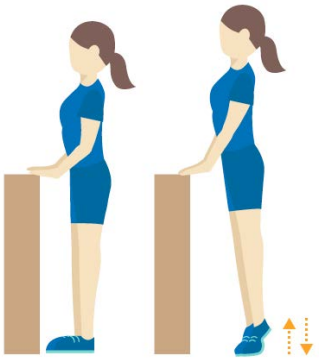
  Stand approximately a foot away from a wall, with your back facing towards it. Rest your back against the wall, keeping it straight. Set your feet shoulder width apart and slowly bend them, keeping your back against the wall. Go as low as you can until you

feel your legs working and hold this for a count of five to start with. You can walk your feet out, further away from the wall, if need be.

Progress this by increasing the amount of time you can hold this for, or by going slightly lower (don't go any lower than knees bent to a right angle).

- **Heel raises (M8)**

  Stand upright behind a chair, and hold onto the back of the chair with both hands for support. With feet slightly apart, push up onto your tip toes and hold for a count of five. Aim for five repetitions.



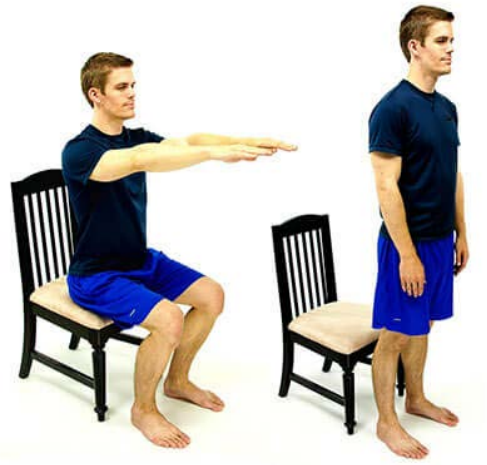
  Progress this by increasing repetitions, or to unassisted to gain improvements in balance, if you feel comfortable.

- **Leg lifts- side and back (M9)**

  Stand upright behind a chair, and hold firmly onto the back with both hands. Lift one leg out to the side, trying to keep it straight, and hold for a count of five. Lower it back to its starting position. Repeat this but lift leg to the back, and hold for a count of five.

  Progress this by increasing repetitions or time holding leg at highest point. Try to remember to keep your stomach in the starting position and to not lean.

- **Sit to stand (M10)**

  Sit on a firm chair (such as a dining room chair). Stand up and sit back down, trying to avoid using your arms, if possible. See how many times you can do this within a 30 second period. If you need to take a rest, that's fine.

  Progress this by seeing if you can reduce the amount of rests you take within a 30 second period. Once you have no rests- see how many you can do in a minute and gradually try to increase that.

- **Ball seated leg raises (M11)**

  Sit on a firm chair and place a ball (about football sized) between your feet on the floor. Squeeze your feet together and straighten them so your legs are straight out ahead of you. Hold for a count of five, and repeat for five repetitions.

  Progress this by repeating repetitions.

Some days you may not be able to exercise to the same level as you have done on previous days. It also may take a while to notice a difference, depending on how fit or unwell you might be. Therefore it can be very helpful to record your exercises that you have completed in the table below, to remind yourself that your activity levels are increasing.

Stability

Stability is a measure of how balanced you feel when standing up and moving around. For some people who are very unwell, their stability can often be quite poor. The exercises listed below (taken from the muscular strength exercises) can help improve balance. Try to do at least one of these per day:

- M6

- M8
- M9
- M10

Your goals and targets:

We ask that you try and think of some goals, to help determine what you would like to achieve from the exercise programme. Ideally, all the goals should be SMART goals (Specific, measurable, agreed-upon, realistic, time-based). To do this, we ask that you think about HOW you are going to achieve your goals, and to think about what will motivate you to work towards achieving them. It is also important to think about having a back up plan, for an example, see below:

My goals are:

Example: improve my fitness

1.

2.

3.

To achieve these goals, I will:

Example: partake in my exercise plan every day, and log my exercise in my exercise diary

1.

2.

3.

Things that might stop me working towards my goals. How will I prevent this/ what is my back up plan?

Example: If I am tired or busy I might not have time to exercise. I will prevent this by breaking my exercise up into segments throughout the day, or by asking a friend/ partner to exercise with me.

1.

2.

3.

I want to achieve these goals because:

Example: I want to be able to take part in social activities with my family

1.

2.

3.

If I achieve these goals, I will be able to:

Example: go shopping with friends

1.

2.

3.

Other thoughts/ notes:

Your exercise diary:

Week commencing:

	Cardio Exercises	Score	Muscular Exercises	Score	Stability Exercises	Score
Monday						
Tuesday						
Wednesday						
Thursday						
Friday						

Saturday			
Sunday			