Implementing a Healthy Eating Strategy after Heart and Lung Transplantation: A Randomised Controlled Feasibility Study

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In the Faculty of Biology, Medicine and Health

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Abstract

Background: Studies evaluating the possible health-promoting effects of sound nutrition in heart and lung transplant recipients are currently lacking. Despite advances in drug treatment and patient monitoring, lifestyle-associated complications such as obesity, diabetes and cardiovascular disease occur frequently. Following transplantation, a low-fat eating pattern is currently viewed as best standard care. However, a Mediterranean diet based on a varied range of fresh unprocessed foods and supplemented with extra virgin olive oil has demonstrated clinical benefit in various non-transplant populations. The aim of this study was to evaluate the feasibility and acceptability of a Mediterranean vs a low-fat diet intervention in heart and lung transplant recipients, and to assess clinical and biochemical outcomes.

Methods: This was a randomised controlled feasibility trial to evaluate a Mediterranean diet supplemented with extra-virgin olive oil, vs a modified low-fat diet in heart and lung transplant recipients at a single centre. In total, 41 clinically stable male and female (median age 55 years) transplant recipients were randomly assigned (1:1) in two separate 12-month waves (n=24 and n=17) to one of these diet interventions. A range of validated food frequency and adherence questionnaires captured changes in participants’ reported eating habits to 6 weeks post-study. Clinical and biochemical analysis was conducted at baseline, 25 and 52 weeks. Telephone and outpatient contact provided a support mechanism to reinforce dietary behavioural change. Caloric intake and physical exercise awareness were discussed, but not promoted.

Results: Thirty nine participants completed the trial (95%). Adherence to both interventions improved significantly at week 25, and was maintained at 52 and 58 weeks. Compared with baseline, waist circumference decreased in both groups at week 25 (p=0.024). A decrease in blood pressure and heart rate occurred at 52 weeks in the low-fat group only. At 52 weeks, higher adherence resulted in significant improvements in fasting glucose in the Mediterranean (<4.8%) and low-fat (<5%) groups. This respective pattern was also observed with total cholesterol (≤9% and ≤7%), triglycerides (≤9% and ≤20%) and IGF-1 (≤9% and ≤15%). A significant decrease in the LDL/HDL ratio (≤12%) occurred in the Mediterranean group only. Moreover, clinically relevant lipid and glucose regulation changes were observed in each intervention.

Conclusions: The implementation of a prospective 12-month Mediterranean or low-fat diet is feasible and acceptable in a heart and lung transplant outpatient setting. Both interventions were positively associated with improvements in lipid and blood glucose regulation and circulating IGF-1. As part of a multidisciplinary framework, these findings offer an additional therapeutic strategy to optimise outpatient care.
**Declaration**

I hereby state that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning. This thesis contains the author’s original work and contains no previously published or written material, unless specifically referenced in the text. The contribution of fellow academics and contributors has been dually acknowledged, including study and questionnaire design, statistical, data and biochemical analysis, and editorial advice.

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Acknowledgements

Research is a journey, and like any journey it requires a destination. However, I am reminded of the words of the great German poet, scientist and philosopher – Goethe. He stated in one often cited quote that “until one is committed, there is hesitancy, the chance to draw back” and goes on to say that once we commit, then a “whole stream of events issues from the decision, raising in one’s favour all manner of unforeseen incidents and meetings and material assistance, which no man could have dreamed would have come his way”. This study has been supported by countless individuals who provided assistance on every level at every stage. It also consolidates my often-cited remark that “we achieve little in isolation”. Therefore, the findings produced in this study, and the effort incurred, belong equally to all involved.

First and foremost, none of this would have been possible without the constant support of my mum – Sharon Whittaker. Never one to force my hand, she has ceaselessly encouraged me to enjoy what I do. Her compassion and generosity know no bounds and are a constant lesson. Secondly, without the help and support of Dr James Fildes, this study would never have gained momentum. I am indebted to his optimistic outlook and altruistic guidance, which epitomises the scientific importance of collaboration. A “chance” meeting sparked an Australian collaboration with Professor Adele Green, without which this study would have been very different. Her experience helped shape this body of work immeasurably, especially in the early stages. As a literary scalpel, Adele helped guide me along the PhD road, even when her busy schedule necessitated otherwise. Thank you. Adele also introduced Dr Kyoko Miura to the study; with her experience in quantifying dietary adherence. Thank you Kyoko, without your help I would not even know where to begin with extracting and analysing the dietary questionnaire data. From a practical aspect, I owe my sincere gratitude to Matthew Pohl. Matt’s culinary assistance was crucial to the success of the study and without his practical help (and support of Professor Stig Steen and the Igelosa Research Institute) and positive input, the study would not have been a patient success. Thank you to Professors Nisar Yonan and Brian Keevil, and the UHSM R&D team, your support was essential and gratefully appreciated. From a numerical perspective, I would like to say a big thank you to Dr Julie Morris for making sense of the results data. Just as integral to this study are the UHSM/MCCIR research team, of which I am a part. I will not write individual names as you should all know who you are – a heartfelt thank you for all your help and support.
A huge thank you must go to the New Start charity for supporting and funding this study, and to the individuals who tirelessly raise the money that fuels research with the ultimate aim of improving patient wellbeing. This brings me to the patients themselves, who are not actually patients, but fellow individuals, the same as you and I. Thank you for taking part and putting the advice into action, you have taught me many things during this research project, not least compassion. In addition, the UHSM transplant team who supported the project from start to finish. The study could not have been completed without the help and assistance of the outpatient nurses and Stef Harris, who diligently care for our patients, and in doing so extended their help to me – thank you.

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Last but not least are my family and close friends who have provided countless cups of tea and kind words over these past few years – love you.
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<td>APC</td>
<td>Antigen presenting cell</td>
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<td>BMI</td>
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<td>Cell cycle inhibitor</td>
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<td>Oxidised low-density lipoprotein</td>
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<td>PTDM</td>
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<td>PUFA</td>
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<td>RCT</td>
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<td>Ventricular assisted device</td>
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<td>WHO</td>
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Introduction

The immune system is responsible for the removal of an estimated 20-70 billion damaged cells on a daily basis. Homeostasis must be maintained in order for this process to be orchestrated efficiently, and our diet provides the necessary building-blocks or nutrients which enable the human body to function optimally; hence the term ‘optimal nutrition’. The late Peter Medawar and colleagues were perhaps the first researchers to explore the role of dietary immunomodulation in transplantation, and in 1979 provided a significant example of how diet can influence transplantation immunity. Their experiments centred on syngeneic mice divided into two groups, both of which were raised using conventional husbandry aimed at minimising infection risk via aseptic conditions (though not germ free). Group A were fed a conventional but autoclaved diet (nutritionally modified), whereas group B were fed the same conventional but unmodified diet. Group B received allografted skin tissue from group A, and conversely, A received tissue from B. Group B experienced graft rejection rates comparable to those encountered in similar experiments [1]. However, group A exhibited significantly shorter graft survival times and transplantation tolerance became more onerous. This experiment clearly demonstrated that mice fed similar, yet nutritionally modified diets, experienced divergent immune modulation that interestingly, was reversible in either direction by switching dietary regimens [2]. A more recent study offers an additional example of the immunomodulatory role of dietary components. The legume Soy (Glycine max), which contains bioactive isoflavones such as genistein, offers both in vitro and potential in vivo immunosuppressive properties [3]. Lewis rats fed an isoflavone-rich soy protein diet one week before cardiac transplantation experienced a significantly extended rejection-free period compared to rats fed casein or casein/isoflavone enriched diets. Intravenously administered genistein (IAG) also extended heart graft survival when compared to control (p<0.001), and provided an adjunctive effect in rats receiving low-dose cyclosporine treatment (p<0.005). Interestingly, there was no improvement in the isoflavone enriched casein group, the reasons for which are believed to be due to the addition of various other constituents found in soy that may improve absorbency [4]. This last point exemplifies the often complex interaction observed between dietary nutrients.

Inflammation lies at the heart of transplant rejection and significantly influences both short and long-term allograft outcomes. Whilst the generation of an acute inflammatory response can serve a healthy function in non-transplanted individuals, its genesis in transplant
recipients may result in allograft injury or rejection. Strategies to modulate low-grade inflammation are therefore of significant potential benefit. Curtailing allograft inflammation in its early stages reduces the likelihood of the immune system mounting a full scale response to signals generated by both innate and adaptive immunity. Mounting evidence supports a role for dietary immunomodulation, though much of the available research centres on isolated foods or constituents, or is animal-based. However, it is important to remember that when consumed in combination, the unification of food derived nutrients can act in either a synergistic or antagonistic manner, thus highlighting the need for epidemiology-based human nutritional studies [5]. As humans, we do not routinely eat isolated constituents, nor do we only eat one type of food, rather we require a balanced and varied diet. Therefore, approaches studying dietary patterns may yield greater understanding in chronic disease [6]. In the context of this study, the aim is to determine the feasibility and acceptance of implementing a novel dietary education strategy in a thoracic transplant outpatient setting. If this prospective study generates positive findings, it offers the potential to update current dietary advice.

The current dietary approach is based on British Heart Foundation (BHF) low-fat guidelines. However, one alternate dietary pattern receiving much scientific interest is a Mediterranean diet (MeDiet). A traditional MeDiet is a pre-1970s eating pattern, exemplified in the olive growing regions of Southern Italy and Greece, and characterised by a varied range of unprocessed nutrient-rich foods [7]. More recently, increased adherence to the MeDiet has been linked to a reduction in all-cause mortality [8] and disease incidence in a range of patient groups [9-14]. The benefits of a MeDiet are not attributable to any single food item or constituent and work through the synergy of its constituent parts. In addition, many of the individual foods and nutrients that make up this dietary pattern do offer quantifiable health benefits. One such group are the biologically active antioxidant polyphenols, ubiquitous to most vegetables and fruits, and exhibited as vibrant pigmentation. Red wine is an example of concentrated pigmentation, and moderate consumption is a component of the MeDiet. Polyphenols are considered perhaps the most bioactive components of plant-based foods, providing antioxidant and anti-inflammatory effects [15-17]. More importantly, it appears that the aforementioned functional properties of polyphenols contribute to the overall antioxidant and anti-inflammatory outcomes demonstrated in various MeDiet studies [18-20]. Whilst this last statement is valid, the real truth may be more difficult to discern in vivo. These plant pigments act on a subtle level, therefore the likelihood is that the antioxidant and
anti-inflammatory properties they deliver may be best achieved by regularly (daily) eating food groups that contain them in their undamaged form.

Other key nutrient groups are the dietary lipids, which are essential to human health, yet lipid composition and intake volume can either detract or contribute to healthy metabolic functioning. A recent large-scale intervention study (2013) conducted by the Spanish PREDIMED group (PREvención con DIeta MEDiterránea) observed the effects of two types of MeDiet; one supplemented with extra-virgin olive oil (EVOO), the other with mixed unsalted nuts. Results demonstrate both diets were associated with a significant reduction in cardiovascular disease risk. It should be noted that the low-fat (control) diet exerted positive benefits on blood pressure, but its effects in this group were clearly not as effective as the MeDiet group [21]; however, adherence to the low-fat guidelines were not closely observed [9]. Furthermore, a recent meta-analysis (2011) conducted by Nordmann et al corroborates similar findings [22]. Therefore, the MeDiet has demonstrated metabolic advantages in a range of patient groups, but only one post-heart transplant hypercholesterolemia-related study has been conducted. Moreover, low-fat eating serves as standard post-transplant health advice, but no specific studies have assessed its effect following heart or lung transplantation. If we consider that the physiological benefits of a MeDiet represent health issues commonly encountered by transplant recipients, for example, CVD, diabetes, inflammation and hypercholesterolemia, it therefore highlights the motivation for conducting a comparative prospective study.
Chapter 1 Heart and Lung Transplantation: Background

At present, heart and lung transplantation remains the only viable therapeutic option for patients suffering end-stage cardiac and pulmonary disease resistant to treatment. Therapeutic interim options are ventricular assisted devices (VADs) that enable critically ill heart failure patients a bridge to cardiac transplantation [23]. For waiting-list lung recipients, extracorporeal membrane oxygenation (ECMO) may offer short-term hope, but bridging with this technique is still in an early developmental stage [24]. The process of transplanting major organs such as a heart or lungs is understandably complex and requires committed multidisciplinary teamwork to optimise patient outcomes. Solid organ transplantation undoubtedly provides hope for life extension, yet it is not without complications. Despite significant advances in drug treatment and patient monitoring, morbidity and mortality continue to remain high following transplantation [25]. Major post-transplant complications include acute and chronic graft rejection, driven by an allospecific immune response. The process of alloantigen detection is elaborate and developed over millennia in response to foreign agents. Subsequently, this culminates as immune system complexity, and in order for organ recipients to impede its multi-pronged attack, it requires a lifelong cocktail of immunosuppressive medication. However, the result is dichotomous. Since their inception, tailored immunosuppressive regimes have proved effective in prolonging graft survival. Unfortunately, their iatrogenic effects can pose significant immune-related problems as normal surveillance is severely disrupted. This results in increased infection and malignancy risk. Moreover, these various agents work through therapeutic toxicity, but this soon impacts multiple metabolic or tissue-specific processes. For example secondary diabetes can develop with the calcineurin inhibitor tacrolimus [26] and chronic nephropathy with cyclosporine, and various others [27].

Inflammation is paronymous to all transplant-related diseases; therefore strategies to prevent or reduce inflammatory processes are essential and likely to offer significant long-term patient benefits, whilst easing socioeconomic burden. Given the plethora of daily drugs required to maintain a transplant patient, further therapeutic intervention to reduce systemic inflammation is probably not the ideal course. However, a large volume of evidence is accumulating in the scientific literature that reports modification of a Western diet can induce wide scale changes in the inflammatory status of healthy individuals. Moreover, these changes also improve clinical outcome in a range of patient groups, including several that
eventually require heart or lung transplantation [18, 28-32]. An additional benefit of dietary modification is that it allows patients to be proactive, offering self-empowering potential; unlike immunosuppressive treatments that understandably require strict life-long adherence. One diet attracting increasing attention is a Mediterranean-inspired eating pattern (MeDiet). A healthy MeDiet is based on unprocessed fruit and vegetable/herb-rich foods with daily amounts of EVOO, red wine, whole-grains, nuts and legumes, and regular portions of fish/seafood. Research demonstrates that adherence to a Mediterranean-inspired eating pattern can potentially alter not only a range of health disorders, but markedly reduce inflammatory status [18, 28-30].

The rationale for advocating a MeDiet following heart and lung transplantation is to assess its health promoting effects by observing changes in routine and non-routine biomarkers and clinical measurements. A primary question must also be; will study participants adhere to new dietary advice and change habits of a lifetime? A low-fat control group will therefore provide the bench mark for current best practice. The reader must be aware that most dietary intervention studies favour cardiovascular research; equivalent respiratory studies are lacking in the literature. This does not nullify the use of dietary intervention following lung transplantation. On the contrary, both heart and lung rejection are governed by inflammation, and immunosuppressive protocols induce iatrogenic side effects and tumorigenesis in a similar manner. Both heart and lungs share an inseparable relationship, and deleterious processes involving one organ inadvertently affect the other. In addition, a primary outcome of the proposed study is to assess the incidence of infection. Studies observing the relationship between diet and infection are sparse, yet there is evidence that omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acid (PUFAs) ratios alter clinical outcomes in *Mycoplasma hyopneumoniae* infected pigs. The results of this study indicate pigs fed an n-3 menhaden fish oil-rich diet experienced the lowest incidence of peribronchial inflammation and lung lesions, compared with the other groups [33]. Whilst this study was not performed in free-living human individuals, it does support the requirement of observing infection incidence in transplanted individuals, as well as other at-risk groups.
1.1. Post-heart Transplant Complications: Overview

The following overview will help inform the reader of the key health issues heart recipients face over the long term. Potential complications are extensive, therefore only the most common issues will be highlighted, and more specific disorders discussed in the following sections. Organ rejection occurs most commonly during the first three months post-surgery. However, late or chronic rejection can still develop over time and one of the primary factors influencing heart graft survival is chronic allograft vasculopathy (CAV). CAV is mediated through innate and adaptive immune responses, which leads to accelerated (cumulative) endothelial damage and subsequent intimal vessel thickening. Resting heart rate following cardiac transplantation is also significantly higher than lung recipients due to the absence of parasympathetic nervous system control, and this can further contribute to CVD development [34]. Nonimmune insults are also implicated and include hypertension, hyperlipidaemia, diabetes mellitus and cytomegalovirus (CMV) infection [35]. In addition to CMV, long-term graft survival is influenced by the continuous threat from other infectious organisms. Immunocompromised heart recipients may be administered prophylactic antivirals and/or alternate therapeutics to counter increased bacterial, viral and fungal infection risk. Therefore, food hygiene awareness is crucial as the gut constitutes a significant region of immune/antigen interface. Accelerated coronary artery disease shares an association with hypertension, dyslipidaemia and elevated body mass index (BMI). Moreover, elevated BMI further complicates long term outcomes as obesity tends to coincide with insulin resistance, which subsequently increases diabetes risk. All of the aforementioned conditions share interrelation and are exacerbated by immunosuppressive regimens. For instance, in a study by Hamour et al, chronic kidney disease (defined by an estimated glomerular filtration rate (eGFR) of <45), occurred at a rate of 45% within one year [36]. The nephrotoxic effects of calcineurin inhibitors are chiefly responsible, but each individual’s clinical situation can rarely be attributed to one distinct cause. Therefore, from a cardiologist’s perspective, outpatient monitoring may at times prove challenging as the potential obstacles are extensive. In addition, immunosuppression significantly increases malignancy risk as immune surveillance is severely blunted. Squamous and basal cell carcinoma rates are also significant, and development of post-transplant lymphoproliferative disorder (PTLD) can prove fatal. Additionally, steroid medication not only dysregulates glucose metabolism, it also contributes to bone density loss, which over the longer term may result in osteoporosis. When viewed collectively, these aforementioned complications can pose a challenge for patients and
practitioners alike and require effective communication strategies to help ameliorate progression.

1.2. Post-lung Transplant Complications: Overview

Lung recipients encounter post-surgical metabolic complications in a similar manner to heart, as the incidence of hypertension, dyslipidaemia, CKD, osteoporosis and malignancy occurs frequently. Unfortunately, median single and double lung survival is the lowest of all solid organ transplants at 4.6 and 7.3 years respectively, as opposed to 11.9 for heart [37]. Management of the lung recipient necessitates a slightly different approach as physiologic changes such as impaired cough reflex can make mucoid clearance problematic. Patients have to remain mindful to periodically cough and thus minimise congestion. It therefore follows that lung recipients are at increased risk of chest infection compared to other forms of transplantation. This differs from heart recipients in that heart tissue is not directly exposed to pathogens. Conversely, airborne bacteria pose one of the greatest challenges to easily accessible lung tissue, hence the routine prescription of prophylactic antibiotics. Fungal spores are equally pervasive and Aspergillus and Candida infections occur frequently, as do community-acquired bronchial viral infections. Both species of organism may also contribute to secondary bacterial colonisation, and vice-versa. The downstream consequences are allograft impairment and heightened rejection risk [38]. Chronic Lung Allograft Dysfunction (CLAD) is the commonly used term for the histological finding of chronic graft rejection. CLAD may be further categorised into Bronchiolitis Obilterans Syndrome (BOS) and Restrictive Allograft Syndrome (RAS), both of which may involve allo and non-alloimmune components. The majority of patients will develop BOS by 5 years and this will progress as inflammatory bronchiole lesions, tissue fibrosis and subsequent airway lumen obliteration [39]. In addition, lung and heart recipient health is significantly influenced by dietary habits; therefore the following section will discuss the implications of current post-transplant dietary advice and associated issues.

1.3. Transplant Nutrition: Current Perspective

1.3.1 Pre and Postoperative Nutrition

Pre-transplant aetiology differs greatly at the UHSM centre and symptoms that befall one individual may not necessarily affect another. It is not uncommon for chronically ill individuals to experience some form of cachexia during the perioperative period. Patients
with end-stage lung or heart disease appear prone to malnutrition and may require increased caloric intake to aid post-transplant recovery [40]. Conversely, an alternate obese profile can occur; in this situation, individuals will be required to make a concerted effort to reduce their BMI before surgery can proceed. Either way, preoperative obesity and cachexia are risk factors for heart [41] and lung [42] recipient morbidity and mortality. The early postoperative period places numerous demands on patient health as surgical tissue injury is extensive, and wound healing requires sufficient nutrition to promote healing. Tissue repair requires an anabolic state, yet during this initial period, stress-induced catabolism is intense, which serves to further disrupt metabolism. In addition, gastrointestinal function, motility and appetite are commonly impaired in both cachexic and obese individuals. Therefore, during the early recovery phase, transplant dieticians try to minimise periods of fasting and re-establish enteral feeding as quickly as possible. The aim is to curtail muscle atrophy and fortified protein drinks are used for this purpose. During this stage, The European Society for Clinical Nutrition and Metabolism (ESPEN) and/or American Society for Parenteral and Enteral Nutrition (ASPEN) guidelines are generally followed. Solid foods are provided as per the general in-patient population until patients are discharged. After this point, dietitian support ceases. However, during the early postoperative phase, individuals with low BMIs will often start to gain increasing amounts of weight. This effect can be even more dramatic in individuals starting with a high BMI. Metabolic demands change during the recovery period, which combined with increased appetite and therapeutic steroids, serve to influence physiology [43, 44].

Two potential problems exist during the post-operative period; firstly, patients with poor appetite, and/or requiring increased body mass, will receive NHS advice. This advice advocates calorie dense foods, including full-fat products and refined carbohydrates, which include sugar, syrup, jam, honey and fizzy drinks. The drawback with this approach is that once individuals enter an unhealthy BMI range, they are expected to switch their high calorie diet and follow the BHF low-fat guidelines. Unfortunately, many individuals find the transition to a healthier eating pattern difficult. Secondly, foods containing sugar [45] and refined grain products [46] may promote systemic low-grade inflammation, and numerous studies implicate their negative metabolic effects in a range of disease states. Whilst outside the scope of this study, it is worth noting that the transplant teams’ efforts to ameliorate inflammation during the early postoperative stage are paramount. Yet the negative metabolic effects of the foods listed above work in opposition to this desired outcome. An alternative
approach may be to tailor dietary recommendations around foods that offer antioxidative and anti-inflammatory properties.

1.3.2. Low-fat diet: Background

The drive to encourage low-fat eating may find its inception in the research produced in the US by Keys and colleagues during the 1970s. Keys established an association between CVD and cholesterol and hypothesised that high fat foods, or more specifically, saturated dietary fat and high serum cholesterol was responsible for increasing US rates of coronary heart disease [47]. This advice greatly influenced health policy making at that time, with the ensuing aim of reducing saturated animal fat consumption, and replacing with polyunsaturated vegetable oils and trans-fats. Successive dietary recommendations tended to advocate high carbohydrate intakes to offset the caloric loss of dietary fat reduction. Invariably, this pattern increased the consumption of refined carbohydrate products, rather than complex, lower glycaemic food sources. The researcher John Yudkin was the first to acknowledge sugar and processed carbohydrate as the main driver of ischemic heart disease [48], but it was Keys’ hypothesis that saturated fat was the main culprit behind heart disease that took centre stage. With an exponential rise in obesity rates following implementation of Keys’ findings, it is only recently that excessive carbohydrate consumption has been acknowledged in significantly increasing the risk of CHD development and progression [49]. Unfortunately, the result was many health promoting and weight loss guidelines were structured primarily around total fat reduction. Current dietary recommendations set by various associations define a low-fat diet with an approximate fat intake of 20-30% of total daily calories. The American Heart Association advocates a reference value of <35% of total daily calories [50], which could still be described as a relatively high-fat intake. Conversely, figures lower than 20-30% are generally categorized as ‘very low-fat’. Proponents of extremely low-fat diets maintain fat intakes above 15% are already too high if individuals want to attenuate, and even reverse, the effects of heart disease [51, 52]. The scientific view for the past 2-3 decades has been that high-fat diets contribute to weight gain. Evidence also suggests that diets high in saturated fat raise low-density lipoprotein (LDL) [53, 54]. High plasma LDL is an associated risk factor for atherosclerosis development and progression [55]. However, research implicates oxidised LDL (oxLDL) as a key atherogenesis mediator [56].
The debate between low-fat and low-carb diets has culminated a widespread view that carbohydrate reduction is more effective for weight loss than fat restriction. However, Hall and colleagues recently demonstrate in a tightly controlled in-patient cross-over feeding study, that calorie for calorie, the low-fat diet resulted in greater body fat loss than the low-carb diet [57]. This study was designed to strictly measure physiologic changes and whether the results are achievable outside of a ward setting was not part of the criteria. In contrast, a study by Hu et al reveals significant weight loss in their free-living low-carb group, compared to low-fat participants [58]. Increasing rates of obesity are the combined result of excessive dietary fat and carbohydrates, therefore daily reductions in both nutrients are likely to offer synergistic benefit if adherence can be met.

General post-transplant dietary recommendations follow guidelines set out by the British Heart Foundation (BHF). Patients are offered a BHF booklet containing a range of healthy eating advice. Yet as we shall discuss, a percentage of the advice contained therein may actually be contradictory. Not all fats are created equal, and as such many individuals are confused as to which are the healthier choices. The rise in PUFA consumption has at the time of writing started to raise questions. From a historical perspective, the large scale extraction and consumption of seed oils is a relatively recent advent. Preceding this, seeds were eaten whole and intact. Polyunsaturated oils once pressed and/or chemically extracted quickly become unstable and oxidise. For this reason, seed oils are often fortified with antioxidant vitamins, post-refining. The BHF provides dietary fat advice by asking individuals to substitute saturated and trans-fats with mono and polyunsaturated fats. Without specific distinction, the BHF advice states “Monounsaturated fats can help lower your LDL cholesterol levels”, which are found in olive and rapeseed oils, plus spreads and certain nuts and seeds. Unfortunately, the choice of processing method for these oils greatly alters the final product. First or cold pressing methods produce the highest quality oils and micronutrient profile. Subsequent extraction involves chemical and/or high temperature processing, thus impairing nutritional value [59, 60]. Research indicates that unlike their unrefined counterparts, these refined products may be less beneficial to health by contributing to lipoprotein peroxidation [61-63]. Interestingly, the BHF advocates polyunsaturated soybean, sunflower and corn oils, plus spreads in its advice. These three oils are encountered primarily as refined products (including rapeseed), and high temperature cooking induces further oxidation and nutrient breakdown [64]. Unfortunately, this is the commonest way these aforementioned oils are consumed. Ramsden et al re-evaluated the Sydney Diet Heart
Study with missing data; results indicate participants consuming n-6 linoleic acid-rich safflower oil experienced increased CVD rates and all-cause mortality than was previously reported. The authors conclude further investigation is required as general cardiovascular dietary advice promotes the use of n-6 rich oils [65, 66]. Moreover, current Western consumption of n-6 and n-3 PUFAs is now in a ratio vastly different to that of previous generations, and which, influenced human genetic heritage. Consequently, n-6 to n-3 intake ratios have shifted from as low as 1:1 to as high as 30:1. The result of this shift contributes greatly to inflammatory processes [67, 68].

On these grounds, the decision was taken to modify the advice offered by the BHF, thereby providing our low-fat study participants with more specific advice on fat/oil quality, purchasing and storage, and use in food preparation. In the interest of the reader, fat intake was not calorically governed.

1.3.3. Mediterranean Diet: Background

The Mediterranean diet has recently attracted much acclaim as a healthy alternative to low-fat or fad diets such as the Atkins diet. Contrary to popular belief, a typical Mediterranean diet (MeDiet) does not exist. When viewed from a historical or traditional perspective, it is comprised of specific food groups that would have commonly been consumed in and around the Mediterranean basin. Therefore, in the context of this paper, we will refer to a traditional MeDiet as one eaten pre-1970s and typified in the olive growing regions of Southern Italy and Greece. The MeDiet is comprised of a variety of fresh/seasonal vegetables and herbs, fruits, nuts/seeds, whole-grains and legumes; fundamentally, a varied plant-based diet.

In the developed world, one of the most significant changes over recent decades is a distinct move away from fresh unprocessed produce, to foods of increasingly refined origin. Taking the example of Crete, it can be observed that rural workers in the 1960s, who primarily ate a fresh wholefood diet, were at significantly reduced risk of CVD than similar workers today [69]. This effect is not specific to the Mediterranean region, many examples exist of how a general move towards processed eating patterns contributes to chronic disease processes [70-74]. Furthermore, adherence to a healthy Mediterranean eating pattern is linked to a significant reduction in all-cause mortality [8] and disease incidence in a range of patient groups. An important point to consider is the MeDiet works through the synergy of its constituent parts. Similar to any wholefood diet, its clinical effects are not attributable to any
single food item or constituent. However, many individual foods and nutrients that constitute this type of eating pattern do offer quantifiable health benefits.

The late nutritional researcher Ancel Keys was perhaps the first person to highlight the health benefits of MeDiet adherence. Keys initiated the long-running Seven Countries Study, which in the mid-1950s aimed to systematically explore the connection between CVD, diet and lifestyle in differing geographical locations. Keys observed that CVD incidence was for instance higher in Finland, where dietary fat intake was high. Conversely, the Japanese diet of the time was typically low in fat and they demonstrated lower CVD rates. Interestingly, Japanese migrants living in Hawaii appeared to develop CVD at a similar rate to their new found neighbours, thus negating a sole genetic advantage. Keys also observed that the population of Crete (at that time) experienced the lowest rates of CVD of all the participants. Incidentally, the Cretans’ ate a diet rich in fat, but its composition was primarily mono and polyunsaturated, comprising olive oil and fish [75, 76]. In the following decades, the distinction between differing oil compositions has been in a state of flux. The scientific view reported during the late 1980s stated that saturated fat intake required curtailing and substituting with polyunsaturated vegetable seed oils. Recent evidence has again rendered current dietary fat recommendations as unclear. Chowdury et al conducted a large meta-analysis and questioned whether n-3 and n-6 fish and vegetable derived oils contributed less to CVD than saturated meat and dairy fat. Their findings indicate a null association between low and high saturated fat consumers and CVD development [77]. These controversial findings may once again serve to further confuse the general public who generally receive scientific results in media sound bites.

Over the last decade, a wealth of positive clinical MeDiet findings has led to a surge in research. In part, this has been conducted to stem the increasing rise in obesity and chronic inflammatory diseases in Mediterranean and Western populations. The Spanish PREDIMED group have recently provided a wealth of positive data from their primary CVD prevention study that has since become the archetype for MeDiet research. The aforementioned study assigned high-risk CVD participants to one of the following three ad libitum diets:

1. MeDiet supplemented with 40ml/day of EVOO
2. MeDiet supplemented with 30g/day of mixed raw walnuts, hazelnuts and almonds
3. Control group received advice to lower fat intake
The results of this comprehensive study were a significant reduction in major cardiovascular events. However, three key points must be considered in relation to the PREDIMED findings; firstly, a combined Mediterranean EVOO and nut diet was not tested. From a tradition standpoint, both food groups were incorporated into the general dietary pattern. Secondly, the low-fat control group did not achieve low-fat status during the study. Total fat consumption at the end of the trial was 37% for control, compared to 39% at baseline. In comparison, final fat intake in the Med-EVOO and Med-nut groups was 41% and 39%, respectively. Low compliance in the control group may be due in part to lack of early dietary counselling; a shortcoming that was amended later in the study. It therefore follows that the PREDIMED study did not observe a characteristic low-fat diet. Thirdly, end of study weight loss in all 3 groups did not achieve statistical significance [9]. Nevertheless, the PREDIMED findings build on those of the Lyon Diet Heart Study, which also demonstrated significant cardiovascular event reduction in patients who had already experienced an MI [78].
Figure 1 displays a selection of food groups that would have been regularly consumed in a Traditional Mediterranean diet.

- **Red wine**: Small glass, 1x per day, sipped with meal.
- **Water**: Drink daily.
- **Varied Daily Intake (each meal)**: Vegetables, fruit, whole grains, extra-virgin olive oil, legumes, nuts, seeds, herbs & spices.
- **Weekly**: Poultry, eggs, cheese & yogurt.
- **Regular (≥twice per week)**: Fish & seafood.
- **Occasional (monthly)**: Red meat & sweet foods.
At the time of writing, studies observing the effects of a MeDiet following heart transplantation are extremely limited, whilst none are available for lung recipients. Salen and colleagues conducted an observation of a French MeDiet on hypercholesterolemic heart recipients. Unfortunately, their study had many limitations, not least a lack of control group and failed to determine positive outcomes [79]. The paucity of post-transplant studies does not negate the potential importance of adopting a prudent MeDiet; on the contrary the wealth of positive studies that now exist when individuals adhere to this type of eating pattern are clinical events frequently affecting heart and lung recipients. More specifically, the benefits of adhering to a Mediterranean-inspired eating pattern are demonstrated in the amelioration of various inflammatory processes [18], that commonly alter long-term recipient health outcomes.

1.4. Transplant Immunosuppression

1.4.1. Gastrointestinal Function

Post-transplant maintenance therapy will typically follow a triple combination strategy, which includes a calcineurin and cell cycle inhibitor and a glucocorticoid such as prednisolone. All three drug classes may individually and collectively contribute to gastric dysfunction by altering GI transit time, microbiota and nutrient absorption. Furthermore, in the early postoperative period, it is not uncommon for heart and lung recipients to experience gastrointestinal dysmotility. This arises due to surgical vagal nerve damage [80] and may require patients take a dopamine-receptor antagonist such as domperidone or metoclopramide to counter symptoms of nausea and/or vomiting. The following chapters highlight the most frequently encountered digestive/metabolic issues observed in clinical practice.

1.4.2. Glucocorticoids

Glucocorticoids (GCs) are widely used to suppress the effects of acute and chronic inflammation and play an integral role in maintenance immunosuppressive therapy. Inflammation is an essential defence mechanism employed immediately following acute tissue injury or infection. However, left unresolved, inflammation can seriously disrupt homeostasis and its genesis following transplantation may result in graft rejection. Therefore, general University Hospital of South Manchester (UHSM) clinical guidelines require patients’ receive a daily oral prednisolone maintenance dose of 0.25mg/kg/day. This
equates to an average single daily dose of 5-15mg; a decision based on previous rejection episodes and general wellbeing. Understandably, steroid exposure should balance between minimal exposure and therapeutic activity. Long term clinically stable individuals are occasionally weaned off therapeutic steroids, and where appropriate, this can prove advantageous as continued exposure is associated with a multitude of co-morbidities [81]. In general terms, prednisolone works by altering leukocyte gene transcription, which subsequently results in suppressing pro-inflammatory cytokine, chemokine and adhesion molecule activity, and leukocyte chemotaxis at the site of tissue injury [82]. Continued use of GCs may seriously disrupt metabolic processes, contributing to the development of hypertension, insulin resistance and diabetes, dyslipidaemia and osteoporosis [83]. The range of documented side effects for steroids is extensive, but within the context of this study, we will highlight those most commonly encountered at the UHSM transplant centre.

Post-transplant weight gain outside of optimal BMI is regularly attributed to GC use, and has been demonstrated in heart recipients [44]. Despite this commonly held belief, supporting evidence for lung recipients is more equivocal, and a recent study by Kugler et al indicates maintenance steroid therapy was not a predictor for increased BMI “for most” patients in their study [84]. In contrast, an extensive review conducted by Berthon et al concluded that long-term use in other disease states may result in significant BMI elevation [85]. GCs are known to disrupt fluid and electrolyte balance, which may in part explain patients’ perception of weight gain. Therefore, salt and high sodium foods are generally restricted. Patients regularly report gastritis-induced nausea symptoms, an effect not solely attributable to GCs and will be discussed in sections 1.4.3. and 1.4.4. Nonetheless, osteoporosis is a common side effect of long-term GC use [86] and to counter this patients may be prescribed bisphosphonate drugs to increase bone density. Unfortunately, this class of drug tends to elicit nausea [87]. GCs are also associated with increased appetite [88], which may be a contributing factor in patient-perceived weight gain. Glucocorticoids are named as such for good reason; glucose dysregulation and insulin resistance can occur in a dose-dependent manner with both short [89] and long-term [90] administration, which may contribute to patient-reported food cravings. In simple terms, by impairing insulin signalling, GCs such as prednisolone can trigger peripheral insulin resistance. Pancreatic β-cells compensate for reduced insulin sensitivity by upregulating insulin production and continued demand can therefore result in postprandial (and fasting) hyperglycaemia [91]. One of the problems with GCs is that morning glucose readings may be normoglycaemic or borderline, but then rise midday after prednisolone ingestion, hence fluctuations may be cyclical. Aberrant glucose homeostasis may require patients to take an oral antidiabetic drug
such as Metformin to aid blood glucose control. Additional strategies may involve dietary adjustment by consuming unprocessed complex carbohydrates to reduce glycaemic load. Furthermore, dyslipidaemia is a frequent complication following heart and lung transplantation [92, 93], and whilst its multifactorial origin in these patient groups is linked to genetic history, diet and impaired glucose homeostasis, evidence suggests GCs may contribute to its development [94, 95]. Normal digestive and metabolic functioning can be disrupted by GC medication, but it is important to remember that transplant recipients combine them with calcineurin and cell cycle inhibitors, amongst others. The aforementioned drugs also contribute their own side effects and these will be discussed in the following section.

1.4.3. Calcineurin Inhibitors

Calcineurin inhibitors (CNI) such as cyclosporine changed the course of transplant immunosuppression by significantly helping to reduce the incidence of organ rejection. Their primary action is to selectively suppress T-cell mediated responses. CNIs are a lifelong recipient requirement and whilst no definitive dose exists (due to metabolic variability) patients are tested regularly in clinic to ascertain optimal therapeutic range. For the majority of patients, cyclosporine represents the first line CNI and is taken twice daily. It functionally binds to cyclophilin, a ubiquitous cytosolic lymphocyte protein, and inhibits calcineurin phosphatase activity. Cyclosporine specifically targets T-cell activation by inhibiting cytokine transcription genes coding for IL-2 and IL-4 [96]. Tacrolimus represents the other main CNI alternative that functions in a similar manner to cyclosporine. Tacrolimus is the UHSM second line CNI of choice when practitioners face persistent recipient rejection episodes. However, there are careful considerations to make before choosing either drug. UHSM post-transplant protocol requires stepwise dosing over subsequent months to achieve optimal target levels of both CNIs. Frequent monitoring helps ensure therapeutic activity is balanced with minimised toxicity. Moreover, drug activity can be significantly affected by various pharmacological agents, and even foods such as grapefruit. The side effects of both CNIs are extensive, and similar to corticosteroids, overlap exists between the two medications, which can further exacerbate problems. Gastric disturbances such as nausea, vomiting, stomach discomfort and diarrhoea are relatively common occurrences, though these effects tend to be dose dependent and are generally reversible [97]. A study by Mayer et al, indicates that tacrolimus-induced diarrhoea occurs more frequently than cyclosporine [98]. Furthermore, cyclosporine-induced hyperglycaemia occurs less frequently than tacrolimus,
but nonetheless there is a propensity for this CNI to increase post-transplant diabetes mellitus (PTDM) risk [99]. In regards to this last statement, UHSM clinical observations are aligned with this view, as are the findings by Ye [100], yet a study by Guethoff (n=60) concluded similar long-term diabetogenic risks for both CNIs [101]. As discussed in section 1.5.3, post-transplant hyperlipidaemia occurs frequently in both heart and lung recipients, yet development in individuals prescribed cyclosporine is higher than tacrolimus [102-104]. Factors for this include CNI medication and preoperative coronary artery disease (CAD), but are additionally influenced by cell cycle inhibitors (CCIs) such as azathioprine, corticosteroid-induced glucose intolerance and dietary fat and carbohydrate intake [105], amongst others.

1.4.4. Cell Cycle Inhibitors

Similar to CNIs, cell cycle inhibitors (CCIs) are generally taken life-long. Mycophenolate Mofetil (MMF) is used first line in heart recipients, whilst azathioprine performs a similar role for lung. When metabolised to mycophenolic acid, MMF inhibits purine nucleotide synthesis pathways required for T and B-cell proliferation, antibody formation and cell-mediated immune responses [106]. Conversely, the prodrug azathioprine is a similar purine analogue that acts to markedly alter T and B-cell DNA synthesis, resulting in immunosuppression. Unfortunately, azathioprine lacks functional specificity; therefore its mode of action is not limited to lymphocyte suppression and this may account for its range of side effects. Azathioprine and MMF can both cause GI disturbances such as nausea and vomiting, but diarrhoea incidence is markedly associated with the latter [107]. In such cases, doses are usually reduced until symptoms abate. In addition, various studies demonstrate negative changes in lipid profile when either CCI is included in immunosuppressive triple therapy. However, conclusive evidence attributing atherogenic lipid profiles for either drug in isolation is lacking. The fact remains that all three classes of triple-therapy drugs greatly alter digestion and metabolism; therefore attributing specific outcomes in combination remains a challenge.

1.5. Post-transplant Comorbidities

Comorbidity incidence is a common occurrence following heart and lung transplantation. Factors that impact long-term patient health are wide ranging, yet the unfortunate irony is they are regularly induced by maintenance drug therapy. The potential list of complications is
too extensive for this body of work; therefore the following section will discuss the clinical conditions that are most relevant.

1.5.1. BMI Influences Transplantation Outcomes

The clinical implications of obesity and cachexia are assessed prior to transplant as standard procedure. Transplant clinicians use BMI as a means of determining nutritional status and body fat ratio using this weight/height\(^2\) index. BMI does not distinguish between lean and body fat mass, as bone density, muscularity, adipose tissue and oedema status can vary greatly between individuals. However, research demonstrates BMI values outside of ideal range are associated with increased morbidity and mortality [108-112] in a range of chronic diseases. Moreover, the interaction between BMI and recipient age may also significantly influence survival [113]. The World Health Organisation (WHO) defines underweight as <18.50, normal range as 18.50-24.99, overweight as >25.00 and obese as >30. An additional three sub-division classes have also been defined for BMI >30 [114]. The association between body mass and patient morbidity and mortality has been noted both pre and post-transplant. Grady et al demonstrated that in heart transplant (HTx) recipients, a BMI favouring obese or cachexic profiles significantly increases rejection susceptibility at 12 months post-transplant [115]. Similar outcomes have been observed with lung transplant (LTx) recipients, whereby at 90 days post-transplant, the risk of death was significantly higher in individuals with a BMI of >27kg/m\(^2\). In this study, there was a trend in morbidity and mortality with BMI values of <17kg/m\(^2\) and between >25 and 27kg/m\(^2\), though the authors state further studies are required to confirm these findings [116]. Nonetheless, this last point has recently been supported as obese lung recipients with a BMI >30 experience a higher 90-day mortality than patients within an 18.5 to 30 kg/m\(^2\) range [117]. Factors influencing nutrient status and heart and lung recipient BMI are; corticosteroid and immunosuppressive medication, and impaired exercise tolerance and mobility, which will be discussed throughout this thesis.

1.5.2. Diabetes

Diabetes is associated with significantly increased risk of cardiovascular and neuropathic complications, and improper glycaemic control and management may severely impact survival [118, 119]. New Onset Diabetes After Transplant (NODAT) has been demonstrated by Ye et al to develop within 2 years in over one quarter of all post-operative heart recipients. In their study, a strong correlation existed between immunosuppressive medication such as
the calcineurin inhibitor tacrolimus, or a BMI >25, both of which are potentially modifiable risk factors [120], albeit to lesser or greater degree. Similar findings have been highlighted in lung recipients. Hackman and colleagues observed 156 patients and demonstrated that within 12-months, almost all subjects exhibited dysglycemia, whilst 32% developed NODAT [121]. Both these studies are significant as they demonstrate the frequency of diabetes development in this patient population. Family history, advancing age, genetic predisposition and ethnicity are non-modifiable risk factors for NODAT. Conversely, >BMI, cytomegalovirus and hepatitis C can potentially be addressed through education and screening. Corticosteroids may also be dose-adjusted in stable individuals, and CIs such as tacrolimus may be switched in some cases to less diabetogenic options such as cyclosporine. Regression of NODAT was demonstrated in a 12-month liver transplant pilot study by Lorho et al, through combined CI switching, anti-diabetic therapy and dietary education [122]. A systematic review and meta-analysis conducted by Heisel also confirms tacrolimus incurs a greater risk of diabetic development than cyclosporine [123]. These findings were confirmed in a large European HTx study; unfortunately, the incidence of dyslipidaemia and arterial hypertension were significantly higher in the cyclosporine group [124]. This last point exemplifies the trade-off transplant clinicians regularly face and highlights the importance of individualised immunotherapy. When viewed from a dietary perspective, a wealth of studies supports diabetes management and regression in non-transplant populations [125-127]. Conversely, there are a paucity of dietary/diabetes intervention studies in solid-organ transplantation, at least one study (n=42) demonstrated improved BMI and metabolic control in heart recipients [128].

What is clear is that heart and lung recipients rarely encounter dysregulated glucose metabolism in isolation; rather, a clustering pattern exists that includes hyperglycaemia, hypertension, dyslipidaemia and increased BMI. This pattern has been termed the ‘metabolic syndrome’ (MetS) and its development post-transplant may significantly impact long-term survival [129, 130]. Research suggests MetS incidence can be significantly reduced by healthy eating patterns, including the MeDiet [131]. Conversely, Yubero-Serrano et al report insulin resistant MetS patients responded favourably to a low-fat, high complex carbohydrate diet supplemented with n-3 fish oil [132].
1.5.3. Dyslipidaemia

Dyslipidaemia is a common occurrence following transplantation, with development and progression posing serious risk for future cardiac events. Its pathogenesis involves elevated serum cholesterol; typically, elevated low-density lipoprotein (LDL) and triglyceride (TG) fractions coinciding with decreased high-density lipoprotein (HDL). Moreover, dyslipidaemia is regularly observed in heart, lung, kidney and liver transplant recipients [133-136]. Research conducted by Ballantyne demonstrates heart recipient plasma LDL, HDL and TG values were significantly elevated in the following 3-months post-surgery [137]. Therefore, lipid-lowering strategies are implemented immediately post-transplant, and in many cases prior to surgery, as aggressive control can greatly improve survival [138]. For transplant recipients, ideal fasting cholesterol and TG ranges appear sparse in the literature, but current The National Institute for Health and Care Excellence (NICE) guidelines (CG181) state high-risk individuals should aim for a 40% reduction in non-HDL cholesterol [139]. In addition, lipid measurements can be used to estimate cardiovascular risk by calculating lipoprotein ratios, which may provide increased diagnostic value over isolated values [140].

As discussed in section 1.1, CAV development in the heart recipient is strongly associated with impaired lipid metabolism. The onset of hypercholesterolemia in the lung recipient has also been documented by Silverborn et al at years 1, 3, 5 and 7, at respective rates of 16, 33, 48 and 58% [141], demonstrating adequate lipid control is essential. Nutritional assessment is conducted early during patient recovery as caloric intake generally increases. Therefore, dietary fat and carbohydrate advice is provided according to BHF guidelines, and unless further specific advice is sought, dietary education ceases soon after. Clinical lipid management for heart and lung recipients involves the use of a statin (e.g. pravastatin) to inhibit the hydroxymethylglutaryl coenzyme-A reductase (HMG-CoA) pathway. Statins are not always effective or well tolerated and may therefore be combined with a cholesterol absorption inhibitor such as ezetimibe [142, 143]. If this combination proves inadequate, statins may be switched to an alternate variety, for instance atorvastatin or simvastatin. Unfortunately, clinicians face the paradoxical challenge of ‘ideal’ medication choice as glucocorticoids and CI inhibitors are implicated in accelerating post-transplant hyperlipidaemia [144, 145].

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1.5.4. Hypertension

Hypertension, as previously noted, represents a significant health risk for both heart and lung recipients. Within 12 months of receiving a donor heart, 71.8% of individuals will develop hypertension and this figure rises to almost 91.7% within 5 years [146]. Conversely, lung recipients exhibit rates of 51.8% and 81.5%, respectively [147]. Its pathogenesis in the heart and lung recipient is a multifactorial process, mediated through sustained systemic vascular resistance, impaired renal function and central mechanisms. The literature is replete with post-transplant hypertension studies; however, relevant lung studies are currently lacking. This appears paradoxical given the International Society for Heart and Lung Transplantation (ISHLT) figures presented above. On these grounds, its manifestation in heart recipients will primarily be discussed, and where possible, contrasted with relevant lung research.

Pre-existing hypertension significantly influences post-transplant progression, whilst donor family history may also contribute a developmental role. Once again, immunosuppressive medication tends to complicate matters as cyclosporine (and tacrolimus) can have a significant effect on blood pressure through sympathetic nervous system activation [148]. A retrospective study by Morrison et al also demonstrated cyclosporine-induced hypertension in lung recipients, whereby incidence was comparable to kidney and bone marrow recipients, yet lower than heart [149]. In addition, fluid retention may further destabilise blood pressure through an impaired renin-angiotensin-aldosterone system (RAAS) [150]. An angiotensin-converting-enzyme inhibitor (ACE inhibitor) may be prescribed for this reason, in combination with alternative antihypertensive drugs, as many individuals fail to respond effectively to a single agent. In fluid retentive individuals, clinicians may offer restricted sodium advice as part of a low-salt dietary plan. Singer and colleagues demonstrate hypertensive heart recipients are sensitive to dietary sodium and restriction may offer a nonpharmacological role in blood pressure management [151]. In general, lung recipients are provided with similar dietary advice, yet specific studies are lacking. Interestingly, a study by Howe et al indicates hypertensive individuals taking ACE inhibitors benefited from restricted dietary sodium, and this effect was potentiated when supplementing with fish oil [152]. Moreover, postoperative heart recipients administered n-3 fatty acids at a dose of 4g per day, benefited from a prophylactic antihypertensive effect [153].
Blood pressure shares an inextricable link with kidney homeostasis; firstly, renal sodium and fluid retention can occur through excess circulating glucocorticoids, with a resulting increase in mean arterial pressure [154]. Secondly, CKD is in part a cause and consequence of hypertension and its incidence in lung and heart recipients is widespread. ISHLT figures report renal dysfunction in one year LTx survivors at 21.6% and 25.8% in heart, with both figures doubling within 5 years [147]. The mechanisms underlying renal-induced hypertension are still not fully elucidated; however, it is known that oxidative stress and inflammation are associated with CKD and hypertension development and progression [155].

1.6. Potential Benefits of Dietary Modification Post-transplantation

1.6.1 Antioxidant-rich foods

Oxygen is a metabolic energy source and an essential requirement for aerobic life, without which, the process of evolution as we know it would not be possible. However, a paradoxical situation exists as biological molecules such as proteins, nucleic acids and lipids are susceptible to oxidative damage. This is especially true of unstable oxygen by-products such as reactive oxygen species (ROS). Exogenous ROS such as toxins and chemicals may seriously disrupt cellular homeostasis, as do endogenous by-products such as those formed through mitochondrial oxidative phosphorylation. It is therefore no surprise that various life forms have developed integral antioxidant systems, for example, super-oxide dismutase or glutathione to counter this degradative metabolic threat [156]. It has been postulated that cellular aging is in part a process of oxidative damage, albeit from a chronological perspective [157]. The significance of oxidative stress (OxS) in the transplant setting is extensive. Perez et al have demonstrated that OxS persists in the heart recipient post-transplant [158], and similar findings were demonstrated by Witman [159]. The role of OxS is just as significant for lung recipients; Behr indicates that post-transplant BOS development is mediated by disproportionate OxS and a paucity of glutathione [160], findings that were previously observed by Riise [161]. Excessive pro-oxidant production is known to usher OxS; conversely, a lack of antioxidants may also drive this process [162]. In order to suppress normal physiology, we may deduce that immunotherapy is by its very nature therapeutically toxic. However, research attributing the deleterious effects of various transplant medications to OxS are essentially experimental and/or animal based [163-165]; though cyclosporine has demonstrated OxS in a human lupus erythematous study [166]. It may be fair to state that the daily oxidative load transplant recipients undergo is extensive; therefore, can it be offset? The field of dietary antioxidant research in cell culture, animal and
Human models are extensive. Human evolution was shaped by our ingestion of these exogenous substances. Yet some of their absorption and mechanistic actions remain an enigma. Dietary antioxidants are found in three main groups, and act either directly, or as co-factors:

1. Vitamins: A, C, E
2. Trace metals: zinc, selenium, copper, manganese, iron
3. Plants: polyphenolic compounds

All three groups work synergistically within the body and deficiencies may significantly impact human health [167, 168]. Vital antioxidants are generally the first nutrients to be stripped during food processing and refining, hence why many of these substances are added as fortifying agents post-refining [169]. It is proposed herein that the action of these substances, especially plant polyphenols, act in a subtle, trickle-like manner when consumed daily. An unprocessed plant-based diet provides the richest source of exogenous antioxidants; coupled with the fact that transplant recipients experience continuous OxS; an eating pattern based on these principles may provide an opportunity to abrogate some of the problems associated with oxidative damage. Polyphenol metabolites can be measured as dietary adherence markers in both fasting urine and plasma samples, which will briefly be discussed in section 3.13.5.

1.6.2. Antibiotics, microbiota and dietary fibre

The discovery of immunosuppressive calcineurin inhibitors helped revolutionise transplantation by reducing the incidence of short and long-term graft rejection. Combined with other immunosuppressive agents, this culminated in improved long-term survival. Unfortunately, regimens incorporating this class of therapeutics are replete with complications, which are discussed in section 1.4.3. Opportunistic pathogens are a key post-transplant concern as recipients interact daily with numerous species. To counter this challenge patients’ may be prescribed antibiotics, either acutely, or as prophylaxis. Similar to cyclosporine, antibiotics such as penicillin also revolutionised modern medicine; however, science now demonstrates that their effectiveness may also be a limitation due to antimicrobial resistance [170]. Antibiotics are not selective in destroying microorganisms; in fact the root word literally translates as ‘against life’. The importance of this last point centres on the GI system as it harbours myriad commensal bacteria. This community of
microorganisms, when in balance, is essential for human health and interacts symbiotically in a number of ways:

- Break down food to extract essential nutrients
- Produce vitamins that human genes are unable to produce
- Intimately involved in immune development and maintenance
- Manufacture anti-inflammatory compounds
- Ward off disease-triggering microbes [171, 172]

The average total number of human cells is outnumbered 10-1 by the bacteria that live in and on us. This commensal community has been termed the microbiome. The newly developed Human Microbiome Project (HMP) aims to characterise their function and diversity in the human population [173]. Antibiotic use significantly impacts intestinal homeostasis, and multiple exposure is linked to increased obesity [174], diabetes risk [175], and insulin sensitivity in patients with metabolic syndrome [176]. Owing to continuous bacterial risk, antibiotic use in transplantation is a frequent necessity. Research demonstrates that the variety and volume of non-digestible carbohydrate (fibre) influences the structure of gut microbiota communities. This ‘prebiotic’ fibre in the form of vegetables, fruit, seeds/nuts and edible fungi provides a healthy grazing environment for intestinal flora [177-179]. Transplant recipients are removed from antibiotic decision making processes; however, they can choose to eat a plant-rich diet that offers the potential to positively modulate intestinal health.

1.6.3. Short-chain fatty acids and Immune Function

As previously discussed in section 1.6.2, intestinal and general homeostasis is inextricably linked to gut microbial health. One area of research attracting much interest is in the cross-talk between gut microbes and innate and adaptive immunity. When in balance, a symbiotic relationship exists whereby the human host benefits from nutrient provisioning of various end products. The most notable of which are the short-chain fatty acids (SCFAs) butyrate, acetate and propionate [180]. SCFA research provides one of the best examples of how intestinal microbes interact with the human diet to influence immune response. Butyrate is the primary energy source of colonocytes and provides an essential function in normal colon physiology. Research has revealed that inflammatory bowel disease is linked to impaired butyrate metabolism [181]. Butyrate’s broad anti-inflammatory action possesses the ability to alter T-cell proliferation, activation, cytokine expression and prevent antigen presenting cell (APC) adhesion. This is important as studies suggest that sustained over-nutrition of fat and refined
carbohydrates is linked to obesity-driven chronic low-grade inflammation [182]. Furthermore, dysbiotic gut flora manufacture endotoxins that may promote chronic inflammation, thus contributing to increasingly common obesity-related disorders such as metabolic syndrome. Xiao and colleagues demonstrated that a dietary protocol comprised of pre-biotic foods reduced inflammation, improved weight loss, insulin sensitivity, intestinal integrity, BP and lipid profile in their 23-week trial [183]. The prevalence of metabolic syndrome in one HTx study was measured at 41.9% within 3 months, whilst Savioli et al state one fifth of their LTx patients developed metabolic syndrome in the year following transplant [129, 130]. This demonstrates the potential frequency of diet-related metabolic disorders within our study population. Moreover, if bacterial gut communities are out of balance, as encountered with periodic antibiotic use in the transplant setting, then the fibre-rich diets encouraged in both intervention groups may provide a strategy to ameliorate some of these deleterious effects.

1.7. Dietary Adherence

1.7.1. Determining Adherence

The ability to accurately determine dietary adherence in free-living individuals remains paramount for intervention studies aimed at assessing the relationship between nutrition and disease. Methods can be subjective, objective or a combination. Subjective measures include questionnaires (closed or open ended), food diaries, 24-hour recall and estimated food records, which are either self-reported or conducted with the help of a trained interviewer. Self-reported subjective measures rely on memory and unless information is recorded immediately, introduce a range of error that must be calculated during validation. Furthermore, an investigator must ensure that respondents are clearly briefed about their requirement to accurately provide/record the food types and volume consumed.

Objective assessment includes the identification of nutritional biomarkers in blood and urine, and weighed food records in an inpatient setting. Dietary metabolites offer the advantage of increased detection and exposure accuracy as they remove the potential bias of mental recollection. This strength is centred on detection of specific compounds and nutrients such as carotenoids and vitamin C, or fruit and vegetable polyphenols. Polyphenols are ubiquitous biomarkers that can provide an accurate measure of plant-based food intakes in both plasma [184] and fasting urine [185] samples. Medina-Remón and colleagues developed a high throughput method for quickly determining the total phenolic content of fasting spot urine
samples for the large PREDIMED trial [185]. The advantage of this method is the ability to provide a general excretion assessment of the most common dietary phenolic; gallic acid. The method is also cost effective and on these grounds was deemed feasible for use in this study and adopted during preliminary analysis (see method section 3.13.5). There are however drawbacks to biomarker assessment. For instance, individual metabolism greatly alters nutrient absorption and excretion, making definitive intake estimation difficult [186]. Moreover, many individual varieties of food do not always contain one specific identifying compound, which makes the ability to accurately detect a combination of commonly consumed food groups a nutritional Holy Grail. Biomarker detection in transplant populations may be potentially challenging due to impaired kidney filtration and immunosuppressive regimens. However, definitive research studies confirming this last point are unfortunately lacking.

Both methods undoubtedly have their own inherent strengths and weaknesses; therefore, we tried to identify suitable methods early in this study. One such method is a validated 4-day food diary, capable of capturing participant information [187, 188]. The aim was to provide patients with a compact food diary to help collate a detailed record of foods eaten over a 4-day period. Further justification for using a 4-day food diary is its ability to focus awareness of an individual’s daily eating habits, but it became apparent that this method may lack suitability [189]. Meetings held with research dietitians at the UHSM Nightingale Cancer Prevention centre highlighted certain limitations. Firstly, participants may not always remember to enter data after each meal. Secondly, analysing each individual diary requires a considerable amount of time per data entry. Furthermore, transplant recipients self-monitor daily tests such as BP or forced expiratory values (FEV), and must remember to take various medications throughout the day. Adding data to a food diary would increase this burden, and missing information could significantly impair study outcomes. However, the decision was made to change the approach of a 4-day diary to more suitable methods (see below Section 1.7.2 and 1.7.3).

1.7.2. Food frequency questionnaire

Nutritional human intervention studies that aim to determine diet-disease associations will require a method of quantitative measurement. One such method is the self-reported, quantitative food frequency questionnaire (FFQ) that was developed as a means of determining habitual food intake for large-scale chronic disease studies. The long-running Nurses’ Health Study developed an FFQ that demonstrates reproducibility and relative
validity of macronutrient intake [190]. Collection of individual food data helps identify commonly consumed nutrients that share an association in promoting disease or health. The questionnaire groups related food items, such as vegetables or dairy, then provides a varied range of individual items within that group (see Appendix D). The respondent is provided with a volume or amount. For example, affixed items indicate a ½ cup, 1 teaspoon, 1 slice, medium potato, and weight in grams or a combination. The aim is to simplify and assist the respondent when making approximations. Questions are answered with a tick mark in a choice of nine boxes that correspond with the approximate number of times each food item and volume are consumed over the preceding 6-month period.

1.7.3. Food adherence questionnaire

A short dietary or food adherence questionnaire (FAQ) is an alternate method for measuring adherence and is generally comprised of 5-40 food item questions. Respondents are required to self-report their habitual intakes, typically as a tick-box answer to a pre-prepared question. The decision to incorporate two distinct FAQs in this study was twofold. Firstly, an FAQ can be tailored to specific foods within a dietary protocol. In regards to data collection and quantification, this offers the benefit of simplicity from patient and practitioner perspectives. Moreover, when conducted during consultation they allow the practitioner to immediately gauge adherence. If reported adherence for specific foods/groups is poor, the practitioner can then focus support in these areas. Secondly, the PREDIMED group validated a 14-point FAQ for their successful MeDiet study. The questions contained key foods/groups habitually consumed within a healthy MeDiet [9], on which our questionnaire was subsequently based (see Appendix E). The same study also utilised a 9-point FAQ for their low-fat control group, but refrained from publishing its results. On these grounds, we validated a similar 9-point FAQ (see Appendix F) to help determine adherence in our low-fat group. Both FAQ versions were conducted at baseline, 25, 52 and 58 weeks.

1.7.4. The Implications of Over and Under Reporting

Human psychology is as varied as the food we habitually consume. Therefore, nutritional studies involving free-living individuals must take into consideration the potential obstacles surrounding accuracy of self-reported dietary intake records. Depending on the methodology employed, the corresponding range of error may be significant as over and under-reporting of data is commonplace. A confounding observation highlighted by Black et al indicates biased misreporting is characteristic of certain individuals, including differing assessment methods,
and over a given time period [191]. Moreover, respondents have been shown to under-report intake of food items such as refined carbohydrates, fat and alcohol. Whilst fish, meat, vegetables, salad and fruit tend to be over-reported [192]. One may infer that our propensity to view objects as ‘good’ or ‘bad’ also influences our view of foods that are regarded as healthy or unhealthy. Interestingly, men are less likely to under-report than women, and this effect is more marked with individuals in unhealthy BMI ranges [193].

Each data collection method must take into account its inherent merits and limitations. When interpreting behaviour the ability of an individual to recall food intake over a 24-hour period will inevitably be much more accurate than recollection a month earlier. The development of the Automated Multiple-Pass Method (24-hour recall interview) was designed by the US Department of Agriculture (USDA) to help determine food intake over the preceding 24 hours. However, whilst a 24-hour recall method may be effective under the right conditions [194], it may not always be appropriate. In order to minimise error, it has been reported that 24-hour recall interviews are best conducted frequently, and include a Sunday, in order to achieve an accurate representation of habitual diet [195]. However, whilst this method could have been incorporated into our study with considered planning, the decision to use a combination of validated (quantitative) food frequency and adherence questionnaires were made for this prospective study. The benefit of a combined adherence questionnaire also allowed for supportive discussion during the 6-month consultation. On these grounds, our study employed two assessment methods; however, the risk of individuals misreporting data in both methods is plausible. Macdiarmid et al state in their paper “misreporting is not simply a nutritionist’s problem, but requires a multidisciplinary approach (including psychology, sociology and physiology)” [193].
Chapter Two: Aims

Post-surgical heart and lung recipients encounter a range of chronic health issues at greater rates than background populations. Weight gain and blood glucose impairment are two primary health issues that significantly impact long-term health, yet clinical measures addressing positive dietary habits are primarily available during early recovery phase. Therefore, a comprehensive dietary support and education programme was implemented to assess feasibility within an outpatient setting, and inform a definitive RCT within this patient population.

2.1 General Aims

a) Implement a Mediterranean and low-fat dietary training programme and assess 12-month compliance feasibility in a group of clinically stable heart and lung transplant recipients.

b) Longitudinally assess and compare the anthropometric and clinico-biochemical outcomes of a Mediterranean and modified low-fat diet in this patient cohort.

2.2. Specific Objectives

1. Determine dietary adherence and retention
2. Determine clinical change in a) key anthropometric and clinical measurements and b) a comprehensive range of biochemical measurements
3. Identify whether Mediterranean or low-fat diets confer specific effects in heart and lung transplant populations
4. Assess changes in weight, body mass index and waist circumference
5. Identify changes in glucose metabolism
6. Identify changes in lipid profile

2.3. Hypotheses

a) Implementation of Mediterranean and low-fat dietary training programmes is feasible and acceptable in a group of clinically stable heart and lung transplant recipients

b) Anthropometric and clinico-biochemical measurements can be used to assess key clinical outcomes after implementation of a Mediterranean and modified low-fat dietary training in this patient cohort
Chapter Three: Methodology

3.1. Source Population

Each UK transplant centre acts as a hub for multiple surrounding counties. The UHSM is situated in Wythenshawe, south Manchester and serves the surrounding population extending from Manchester (Lancashire) to Cheshire, Merseyside, North Wales, Derbyshire and Cumbria. UHSM transplant patients living within the Greater Manchester area were considered eligible for the study.

The UHSM transplant outpatient unit operates with a dedicated team of staff, which include nurses, doctors, dietitians, psychologists, physiotherapists and social workers. All efforts were made during study development to build a professional rapport with many of these individuals as their help and support was essential for recruitment and sample collection. This was achieved by daily networking and PowerPoint seminars (including proposal and data collection typically lasting 30-40 minutes), which were arranged on multiple occasions to ensure the transplant team were fully aware of the study. In addition, one to one meetings were conducted. In many instances, informed nurses and doctors would introduce the study to their patients in advance when dietary considerations were discussed. Consequently, advice from transplant dietitians highlighted potential problems that may be encountered, for example, immunosuppressive medication and impaired glucose regulation and digestion.

3.2 Participant Selection and Recruitment

Participants were selected and recruited in 2 identically-planned waves six months apart.

3.2.1. Newsletter Promotion:

The New Start charity provides all Wythenshawe transplant recipients with printed periodic newsletter updates, which contain a wide range of interesting transplant-related articles. Two separate pieces were prepared during this study period. The first article introduced the investigator and highlighted the benefits of regularly eating a fruit and vegetable-rich unprocessed diet. The second letter was aimed at introducing the AMEND-IT study, thereby propagating enthusiasm for potential recruitment. Publishing these short letters proved helpful not only in garnering patient interest, but as a useful means of attracting enquiries from members of the transplant team.
3.2.2. Pilot testing:

A small number of heart and lung recipients were approached with an early version of the recruitment letter in order to gain prospective feedback from a patient’s perspective. Two proactive individuals (one heart and lung recipient) within the transplant community were contacted. Feedback from both parties provided useful points that were taken into consideration when developing the study documents and overall structure. In addition, it was arranged for the investigator to observe a Transplant Support Group meeting to gain insight into some of the physical, psychological and emotional implications of receiving an organ transplant and introduce the investigator and the proposed study. Most individuals in the Transplant Support Group offered their personal opinions, which were also taken into consideration in establishing the study.

3.2.3. Inclusion and Exclusion Criteria and Pre-screening Process:

Eligible study participants were clinically stable heart and lung recipients over 16 years of age with a minimum post-transplant period of 6-months. Capturing newly listed individuals would minimise secondary complications that frequently develop over time. Individuals had to be free from episodes of rejection or infection, cancer, diabetes or chronic kidney disease (CKD). Transplant recipients are pre-disposed to CKD development, owing directly to maintenance immunosuppressive medication. Impaired kidney filtration is detected by quantifying creatinine clearance with an estimated glomerular filtration rate (eGFR). Discussions held with transplant clinicians helped guide the decision to use an eGFR cut-off value of 30. It should be noted that an eGFR reading of 45 or higher was the ideal lower limit in regards to reducing study drop-out rates. However, this was not always achievable. Recent trends indicate that as transplant eligible patients encounter increased suitable organ donor waiting times, the cumulative effects of disease progression and nephrotoxic medications has a greater negative impact on baseline renal function prior to transplant. Low eGFR rates necessitate dietary potassium restriction, which would automatically require patients in both MeDiet and low-fat groups to curtail the high intake of potassium-rich fruits and vegetables advocated in this protocol.

An exclusion BMI limit of 30 was set, based in part on the evidence of high BMI and reduced MeDiet adherence. High BMI is a known predictor for a range of common chronic diseases such as CVD, diabetes and cancer [196-199]. Conversely, increased BMI has been linked to low adherence of a Mediterranean eating pattern in free-living individuals [200, 201]. The
WHO characterises obesity with a BMI >30kg/m2 [114]. The aim was to reduce attrition in this pilot study; though it should be noted that steroid medication tends to elevate BMI scores, thus leading to potential exclusion of participants wanting to make positive lifestyle changes. As participants had not yet consented for AMEND-IT, it was more ethical to review this decision on an individual basis.

Patient records were therefore diligently pre-screened for any of these major complications before contact. Further screening parameters were applied for food allergies or prescribed diets that would deviate from our proposed protocol. Additional inclusion/exclusion criteria centred on 4 specific nutritional questions that determine whether patients are willing to regularly eat specific food groups, or remove them from their diet. Perhaps more importantly, were they willing to try? Similar questions have proved effective in a recent large-scale MeDiet study [9]. Any individuals not meeting these primary inclusion criteria were securely logged to avoid future study contact. Eligible candidates who received a patient information sheet and were potentially interested in the study could then request further information before providing signed consent.

3.2.4. Postal Recruitment:

A list of eligible candidates was compiled and a total of 63 individuals were contacted for recruitment in wave one and 58 for wave two. Envelopes were prepared and contained a general information sheet explaining the importance of following a healthy diet (see Appendix), and a return form with contact details stating whether individuals were interested in enlisting or not. A patient information sheet was also included, along with a return envelope. For patients expressing interest or requiring further information, telephone calls were made to introduce the investigator and answer questions in more detail.

3.2.5. Randomisation:

Following postal recruitment, those heart and lung recipients meeting our inclusion criteria were carefully stratified in order of heart, lung and operation date, with the most recent listings at the fore. Dr Julie Morris, head of UHSM Medical Statistics, was provided a list of codes designed to stratify lung and heart recipients into Mediterranean and low-fat groups. Patients were then randomly assigned to either MeDiet or Low-fat intervention groups using a computer-generated allocation sequence with random block sizes and an equal 1:1 allocation ratio. Randomised codes were sent to a fellow researcher who numbered and
sealed each envelope before storing securely until requested individually at each baseline consultation. To blind the investigator from recruitment bias, sealed opaque envelopes were affixed a sequentially numbered heart or lung label, and defining characteristics withheld.

3.3. Baseline Data Collection

3.3.1. Weight and Body Mass Index

Weight was measured in kilograms using calibrated Seca 877® mobile weighing scales. Footwear, excess clothing and personal items were removed prior to weighing. Height was measured in millimetres with a mechanical wall-mounted Seca 222® stadiometer and footwear removed. BMI was calculated as weight (kg) divided by height (metres), and rounded to the nearest tenth.

3.3.2. Waist Circumference

Waist circumference is a well reported predictor of morbidity and mortality. Measurements were recorded with a European stretch resistant waist tape measure and taken with patients standing upright and clothing removed from the abdominal area. Restrictive clothing items such as belts or tight trousers/dress were loosened before measurement. The tape was placed midway between the iliac crest and underside of 12th rib. Readings were taken at the end of relaxed expiration and measured in millimetres.

3.3.3. Basal Metabolic Rate

Basal metabolic rate (BMR) estimates resting metabolic expenditure in a fasting, post-absorptive state. BMR was calculated using the metric Standard Harris-Benedict Formula.

3.3.4. Blood Pressure and Heart rate

Peripheral blood pressure (systolic/diastolic) and heart rate were recorded using a calibrated Dinamap Pro100V2® device. Readings were taken following a minimum 5-minute rest period. BP cuff was applied to a supported bare left arm with monitor screen turned away from patients view. Measurements were recorded for each individual and stored in their case file.
3.3.5. Blood Sample Collection and Storage

Ethical approval was granted for a 10ml blood sample (at all three time points). Due to difficulties surrounding transplant patient venepuncture, the decision was taken for UHSM outpatient nurses/phlebotomists to collect samples for this study. Liaising with outpatient nurses was fundamental as clinics are generally busy and informing nurses in advance of patient arrival helped ensure additional blood samples were collected correctly. Samples were collected via venepuncture using 4ml EDTA (purple), 5ml serum gel (gold) and 2ml fluoride oxalate (grey) BD Vacutainers®, and inverted as per BD guidelines. Samples were immediately transferred to a 4°C refrigerator before processing. A laminated sample storage chart was prepared and used as a checklist for volume, quantity and analysis method. Serum, plasma and fluoride oxalate samples were individually aliquoted and labelled. HbA1c were analysed the same day, all other samples were stored in a Sanyo VIP series™ -80°C freezer, with T-Scan wireless 24/7 temperature monitor.

3.3.6. Baseline Consent Visit

Participant files were prepared individually and labelled in advance to correspond with either a MeDiet or low-fat random assignment. Each file contained the following:

- Screening questionnaire
- Consent form
- Study checklist comprised of visit documents 1-3
- Questionnaires for each specific group
- Phlebotomy request form
- Defining research labels for patient medical files

Eligible candidates were contacted and dates arranged for each baseline consultation, with a request to fast (no food or liquids [water permissible]) starting at the previous midnight. The reasons for which were risk of sugar, antioxidant polyphenols and milk fat intake impairing biological results. During consultation, each individual was introduced to the study and given all necessary information, and allowed time to ask questions about their involvement. Those interested in enrolling were provided a screening questionnaire and consent form (see Appendix C). Two photocopies of the original consent were taken; one for the patient and the other two for each patient’s medical file and study file respectively. Patient medical files were updated the same day in accordance with trust and research ethics policy. Anthropometric
measurements and participant and questionnaire data were entered into relevant spreadsheets on the same day, whenever possible.

3.3.7. Participant Tracking

Accurate recording of participant data was vital in order to effectively monitor and co-ordinate multiple appointments and clinic visits. An AMEND-IT subject tracking system was set up in Microsoft Excel® allowing the following data to be clearly displayed in table format:

- Subject identification code, name and RM2 number
- Patient information sheet postal date
- Consent, randomisation and baseline data
- Training seminar date
- 25 and 52 week appointments and outcome section

The subject tracker was programmed to calculate due appointments with an adjacent column for actual arrival dates, as amendments were commonly made. Working printed calendars helped plot appointment dates in advance and a Microsoft® compatible smart-phone calendar was utilised to remind the investigator of various contact requirements.

3.4. Trial Design: Nutritional Interventions

Nutritional studies often present challenges such as attrition and adherence, due to the complex relationship humans share with their food. On a daily basis, our requirement of sustenance brings with it inextricably linked issues ranging from emotional requirement to evolutionary mechanisms programmed to seek energy rich foods such as fat and sugars, which calorically speaking are often unused. Therefore, if a dietary modification strategy is to work effectively, it must pay close attention to participant behaviour and attitudes towards food; whilst providing dietary flexibility and regular participant contact with the aim of improving commitment and motivation [202].

3.4.1. Mediterranean Dietary Education

Patients randomly allocated to the MeDiet intervention arm received specific information that encouraged adherence of a predominantly unprocessed eating pattern, representative of a
traditional Mediterranean diet. Participants were required to attend a single MeDiet-based training session at the UHSM outpatient centre following their baseline consent visit (see below Section 3.5). For reasons of support and adherence, patients were encouraged to attend with a relative or person living in the same household. Information provided in this session defined the foods (and food groups) that constitute a traditional MeDiet, for instance:

- Unprocessed food preparation
- Daily intake of a variety of fresh vegetables, fruit, wholegrains and legumes
- Regular consumption of oily and white fish (minimum of 3 times per week)
- Extra virgin olive (40ml per day)
- Small daily handful of raw unsalted nuts (walnut, almond and hazelnut etc)
- Alcohol consumed as red wine with an evening meal (maximum of one small glass per day)
- Occasional red meat consumption, favouring lean poultry instead
- Minimal consumption of sweets/sweet baked goods, desserts, sweetened beverages

The scientific rational for advocating this diet was explained in contrast to the increasing rate of chronic inflammatory diseases. Furthermore, it was discussed how distinct areas of the Mediterranean who still follow this traditional dietary pattern experience a significantly better average life and health span; as opposed to surrounding locations that have deviated from this norm. The training session was divided into two mutually inclusive parts. The first half (see Section 3.5.5) provided the background and science in a visual and interactive manner. Various fresh foods were used as stage property to aid communication and define PowerPoint information. The second half (see Section 3.5.6) of the training session was conducted with the help of Matthew Pohl, a trained chef specialising in healthy food preparation at the Igelosa Life Sciences Centre in Sweden. His role was to demonstrate a range of practical methods for MeDiet application.

Participants were provided with a printed (bound) A4 handbook containing advice on shopping, food preparation and hygiene, storage, dining out and recipes (see Appendix I and J). A 5 litre carton of EVOO was supplied to each participant as a gift to take home. Following this, participants were to receive further advice and support at the UHSM outpatient clinic and periodic telephone consultation (see Sections 3.8.1 and 3.8.2 respectively).
3.4.2. Low-fat Dietary Education

Standard NHS information is based on BHF guidelines; however, their recommendations do not clearly delineate what constitutes a healthy dietary fat, as opposed to processed oxidised versions that are commonly consumed and widely marketed. Heart and lung recipients assigned to the low-fat training group were therefore presented with revised guidelines for reducing dietary fat intake. The composition of poly, monounsaturated and saturated fats were discussed in relation to current science, and healthy populations that restrict its dietary inclusion. Moreover, since the low-fat diet inception, advice has typically been offered in tandem with a high carbohydrate intake, which is now known to contribute to weight gain and various health problems. On these grounds, we discussed the implications of reducing fat and refined carbohydrate intake. Participants attended the low-fat specific training session following the same basic criteria as the MeDiet group. Key information provided for this group included the following:

- Background to what constitutes a low-fat diet
- Define the three main dietary fat types
- Discuss the processing/production of vegetable oils and spreads
- Oxidation of cooked vegetable oils and its effect on health
- Choosing lean cuts of meat, poultry and white fish
- Refined carbohydrate intake and body fat

Background to a low-fat diet was discussed with the aid of a low-fat PowerPoint demonstration. Foods applicable to this group were again used as stage props to help define specific aspects; for example vegetable spread contents. The cooking demonstration incorporated methods for reducing fat intake, whilst providing ideas for flavourful low-fat salad dressings and sauces etc. Similar to the MeDiet session, it was important to reiterate that regular small changes are easily achieved with conscious effort and may be more conducive to long-term change.

Each participant received a printed (bound) A4 handbook prepared in a similar manner to the MeDiet group. However, instead of receiving an olive oil gift, a recipe book was provided (see Section 3.7.2 and Appendix K). Outpatient advice and support included the same methods as discussed previously (see Section 3.4.1 and 3.4.2).
3.5. Patient Education Programme

As previously discussed, both MeDiet and low-fat arms received tailored advice and support, starting with a background of their respective diet group. Basic training for each arm was held over four consecutive days, yet subjects were only required to participate in a single session. Day one (Thursday) and three (Saturday) were held for MeDiet training and day two (Friday) and four (Sunday) for low-fat. The rationale for conducting sessions in this manner offered the following advantages:

- Participants were accompanied (in general) by a partner/relative or close friend. Providing flexibility for weekday or weekend sessions helped ensure persons in employment or with prior arrangements were adequately catered for.
- Spacing sessions over 4 days allows for manageable sized groups (based on proposed figure of 80 participants).

The programme was conducted as a holistic educational package as patients are rarely offered a comprehensive understanding of ‘why’ nutritional changes should be encouraged and adopted. Furthermore, practical advice for achieving these goals are often exempt, which may leave individuals lacking the confidence to initiate new ways of shopping, cooking and eating. This study sought to encourage long-term change in dietary habits through behavioural modification. Therefore, we offered a layered approach to guide individuals (and close family members) with a range of new ideas and provided printed information to accompany the course. The scientific background was provided in an interesting yet understandable manner and was complimented by a trained chef who helped deliver pragmatic advice (see below Section 3.5.6).

3.5.1 Printed Dietary Information and Guidelines

Printed guidelines were an essential component of this dietary intervention study. Complementary literature underpinned the verbal and practical information provided during tuition sessions that may have easily been forgotten. Clear and concise printed information helped convey aspects of the study that were deliberately withheld during the training seminar due to time constraints and risk of information overload. Material provided to both low-fat and MeDiet groups was delivered as a printed and bound booklet (see Appendix I and J). Essentially, the contents were very similar as the basis of the study was to encourage participants to cook unprocessed meals, which predominantly require the daily preparation of
vegetables, wholegrains, fruits and legumes. Specific information was tailored to each group, such as recipes or low-fat guidelines. The low-fat group received a separate recipe cookbook, kindly gifted by Oriflame™ and the Igelosa Research Institute, whereas the MeDiet group received a range of recipes inside their bound booklet. All patients involved in the study were immunocompromised, therefore each booklet contained food hygiene and storage information to highlight the dangers of improper food hygiene.

3.5.2. Facilities and Cooking Equipment

The general facilities required for the study were available at UHSM, the Northwest transplant hub to which many patients travel considerable distances. Holding training sessions at this familiar location ensured the situation was user-friendly for both patients and staff. A seminar room close to the transplant centre outpatients department was identified as an ideal location to hold the consecutive 4 day training sessions, with space for 35 individuals and a screen projector with PowerPoint function. Access to a nearby canteen room large enough for food preparation and washing up was available. Toilets were closely situated, and printed signs were produced to highlight the route. Necessary steps were taken to ensure the environment was safe and fit for purpose, and a fire officer was contacted to determine the legalities of using the seminar room for the study. Cooking facility stipulations highlighted no naked flames and this warranted the purchase of two induction hobs. As the study was charity funded, costs were minimised wherever possible and this required self-providing or identifying available Trust equipment. A meeting was held with a UHSM Sodexo manager to discuss the temporary availability of surplus stainless cooking equipment and disposable items such as bowls, plates and cutlery. The aforementioned equipment was available to loan for each study group and a range of additional equipment and utensils was provided by the investigator. Nylon chopping boards and disposable table covers and napkins were purchased. Hot and cold beverage facilities were identified and duly sourced, whilst a large fridge/freezer was arranged for perishable food storage.

3.5.3. Food Shopping

A key component of this study was providing a practical approach to the tuition seminars and this also applied to food ingredients sourced for the training sessions. Food items that were difficult or expensive to obtain would make it impractical for individuals and family members to follow the dietary recommendations. Therefore, goods were purchased from a range of commonly encountered stores. Food items were attained in bulk for the 4 day
sessions; following this, used/missing items were collected at the end of each tuition day if required. The first visit of the day was to an indoor market with a fruit and vegetable supplier to demonstrate the range of items available at respectable prices. Subsequent visits were made to well-known superstores for other goods and missing list items. The only deviation from this pattern was a visit to a health food store renowned for its fair priced range of dried pulses, grains, herbs and spices. This was to demonstrate that other options are available for non-standard items if individuals were prepared to diversify. Many of the food items were used as educational tools. Most food items were applicable to either group, though nuts/seeds and EVOO were withheld for the low-fat groups.

3.5.5. Group-specific PowerPoint Training

PowerPoint is an effective visual communication tool and the adage “a picture says a thousand words” was heavily factored into this area of the study. The aim was to simplify the science and deliver coherent advice to all participants involved, yet not too simplistic as to lack depth and substance. As our primary recommendations were based on unprocessed food consumption, the core message delivered to both groups was in essence very similar. This essentially allowed the construction of both PowerPoint talks to be the same, albeit from a foundational aspect (see Appendix L and M). The respective PowerPoint talks differed primarily in the following ways:

- MeDiet participants were encouraged to consume daily amounts of EVOO and raw unsalted nuts (e.g. walnuts, almonds, hazelnuts).
- An inverse traditional Mediterranean food pyramid containing key nutritional groups was constructed and explained.
- MeDiet antioxidant food groups were discussed where applicable.
- Low-fat participants received specific slides on ways of reducing fat intake.
- The process of fat/oil degradation during processing, heating and cooking was explained.

Text was kept to a minimum throughout the slides to help maintain participant concentration; each PowerPoint slide/image helped explain a story that aimed to build on the last. Therefore, lively pictures were chosen (or constructed) to exemplify key points, with many used as analogies. Patients and family members were encouraged to interject and ask questions if something required further explanation. Key topics were discussed in a ‘common sense’ manner; for instance the food we eat should provide the primary building blocks for optimal
cellular function. Conversely, nutrient deficient foods impair the body’s ability to heal effectively. The benefits and side-effects of immunosuppressive drug regimens were contrasted with the respective qualities of processed and unprocessed diets. For instance pharmaceutical drugs increase daily metabolic demands and empty calories further contribute to this process. Alternative slides encouraged the audience to think about food from an evolutionary perspective.

Inflammation and its related diseases were discussed in basic terms and linked to modern processed and overconsumed diets. Free-radicle production and antioxidant activity was explained in a jargon-free manner and the function of vitamins and minerals, and their relationship as co-factors for enzyme production. As previously highlighted, many of the food items were used as stage property to exemplify key points throughout the talk. Colourful fruits, vegetables, pulses, grains, nuts, seeds and herbs and spices, along with various other dried/minimally processed goods were laid out for visual impact. Organoleptic testing was encouraged by allowing participants to taste and smell various samples. For example during the talk:

- Olive oil of high and low quality was circulated for the audience to smell and taste, whilst explaining the corresponding health properties.
- Various spices were freshly ground and distributed so the audience could smell the volatile components that offer both taste and reported health benefits.
- Raw nuts were cracked open and their health properties explained.
- The colours of fresh fruits, vegetables, herbs and spices were highlighted as beneficial antioxidant pigments.

The advantage of delivering an inclusive session inadvertently encouraged discussion between the whole group, as individuals remarked between themselves on their thoughts and feelings. By conducting smaller group sessions the overall dynamic changes and this can provide positive re-enforcement of ideas and encourage people to try new things. The ethos of the study was the notion of reprogramming unhealthy habits and daily patterns, thereby supplementing with positive new ideas and ways of thinking about food. At the end of this session, time was allocated for beverages and toilet breaks before starting the cooking demonstration. Hot and cold drinks prepared early in the day were freely available throughout morning and afternoon sessions.
3.5.6. Chef-Guided Training

The assistance of a trained chef working as part of a transplant-based nutritional research team was sought. The chef’s input was essential, and regular Skype meetings allowed mutual ideas to culminate in an effective strategy. Equipment, facilities and ingredient lists were duly compiled over a period of months. The practical knowledge provided by the chef strengthened the study and was essential during the training sessions. A comprehensive range of ideas was compiled, ranging from cost effective shopping, seasonal produce, meal planning, and preparation and storage to provide meals quickly on days when patients feel unwell and lack motivation. The chef visits were scheduled as two one week blocks. A food item list was constructed before arrival, with one day dedicated to food purchase.

The chef’s approach was to encourage the preparation of unprocessed food by talking about the widely available range of fresh produce. He encouraged individuals to add a range of bright colours to their plate at each mealtime by increasing the ratio of fresh vegetables at the expense of processed carbohydrates and meat portions. No-nonsense food was exemplified throughout, thus minimising excessive kitchen time. The act of trying new foods was reiterated throughout the talk, as well as modifying an individual’s current habits and supplementing with healthier options. Foods were exhibited during the demonstration for people to smell and/or taste, adding to the inclusive ethos of the sessions.

Lunch provided a cooking demonstration opportunity with food freshly prepared in front of the participants. Each preparation step was explained clearly, whilst discussing ingredient health benefits. The session was kept open so people could ask questions and interact. By preparing a variety of lunch options, multiple ideas and preparation techniques could be explained. Salad preparation figured strongly and was tailored to each arm by including a range of dressings made with various ingredients. Wholegrain varieties were discussed, in conjunction with preparation methods and batch cooking for subsequent quicker meals. In addition, the merits of minimally processed tinned and packaged items were highlighted. A brief overview of food hygiene was presented to complement the printed handout information. To inform the reader, the chef wove his own personal story into each session as his past health concerns were significant, and overcome by considerable dietary modification.
3.5.7. Training Seminar Survey

An anonymised survey form was prepared to gauge the response of the training sessions (see Appendix H). The form was a simple tick-box format with questions designed around the two halves of the session, and its overall merits. Individuals were asked to answer each question as appropriate and provided an additional comments section for more detailed feedback. The form was designed to influence future studies, and in the interest of study bias, was not used to sway the second study arm.

3.6. External Tuition session

3.6.1. Home Visits and Media Footage

All endeavours were made to ensure patients and accompanying family members could arrive at the dates set for each of the four consecutive dietary tuition days. Whilst we managed to achieve this for the wave two groups, two separate individuals were unable to attend the year one session due to pre-booked holidays. This scenario had already been factored into the study and on these grounds a camcorder had been set-up to pre-record the second half of both MeDiet and low-fat chef training sessions. DVDs were provided to each of these individuals at their home tuition session. The decision was made to carry out home training sessions for the two absent individuals as they had met our inclusion criteria and were already recruited. A PowerPoint presentation was held in a similar manner to the group sessions, and a large range of foods and equipment were used to exemplify certain points and ensure the boundaries between the hospital-based training sessions differed minimally. Both individuals were accompanied by a close partner.

3.7. Adherence Gifts:

3.7.1. Olive Oil

The proven health benefits of extra-virgin olive oil (EVOO) have attracted much attention; however, not all EVOOs contain appreciable amounts of beneficial phytochemicals. Conversely, a high price tag is not necessarily indicative of superior product quality. On these grounds it was decided that in the interests of both patients and the study, a specific quantity of EVOO would be gifted to each member of the MeDiet group to help ensure the following:
1. High polyphenol content
2. Each MeDiet participant consumed EVOO of a similar quality
3. Subsequent EVOO purchases must be easy for participants to source and competitively priced.

It was important for participants to learn what constitutes quality oil versus low-grade products. By networking with various researchers and EVOO experts, we identified a Greek brand named Illiada™ that would ensure the specific aims listed above were achieved. Each participant in the MeDiet group received a 5 litre tin of Illiada EVOO, which depending on family size, was estimated to last up to 3 months at the stipulated 40ml/day intake. Continued use of this brand was encouraged after the initial gift was consumed, though no mandatory stipulations were made.

3.7.2. Low-fat Cookbook

In contrast to the MeDiet gift, the low-fat group received a cook book containing a range of low-fat recipes. This book was written by the contributing AMEND-IT study chef Matthew Pohl and copies were kindly donated by Oriflame™ and the Igelosa research team. The book contains 40 recipes from the Igelosa research kitchen, which follow low-fat principles. In addition, the book is interspersed with preparation sections for whole-grains, salads, alternative complex carbohydrates and various interesting hints and tips to create delicious meals.

3.8 Follow-up and Support Strategies

3.8.1. Outpatient follow-up:

Outpatient follow-up appointments with the investigator were set in the protocol at 6, 25 and 52 weeks, but later changed to omit the 6 week visit. This decision was based on logistical considerations for participants visiting the clinic, as discussed in section 3.1. Clinic visits at week 25 provided an opportunity for face to face contact, thus offering a more personal means of engagement. Whilst all endeavours were made to see patients at routine clinic appointments, this was not always possible. Therefore, in approximately one third of cases, a non-routine appointment was made. The ability to liaise with clinic staff was crucial as rooms equipped with calibrated BP monitors and weighing scales are at a premium. Transplant recipient outpatient schedules commonly involve X-ray, biopsy, lung function, and echo and
electrocardiography measurements. These routine tests had to be circumnavigated if scheduled to coincide with our study visit.

Outpatient nurses and phlebotomists were notified in advance of participant arrival, thus ensuring blood sample collection during routine venepuncture. It was necessary to highlight the importance of adequate BD Vacutainer® filling as staff rotas changed regularly. Inadequate collection would potentially require a clinic re-visit. Fasting urine samples were also collected at each clinic visit, whereby blood and urine sample date, time and patient code were added to a specific diary. The diary proved invaluable for retrospective observation during sample analysis. Each outpatient visit provided an opportunity to discuss re-programming of habits, family support and changes that were both challenging and more easily attainable. Current relevant research findings and complementary information was utilised where applicable. Clinical measurements and questionnaires were conducted similar to baseline data collection.

3.8.2. Telephone Support and Tracking:

Telephone consultation was conducted periodically throughout each 12-month study period. Various studies confirm regular telephone contact can improve motivation and protocol adherence [203, 204]. The aim for this study was to use regular telephone conversation as a support tool for encouragement with the ultimate goal of improving compliance. Initially, each telephone consultation was allocated 15 minutes. This was later adjusted to compensate for the significant amount of time required to consult 80 patients (initial proposed figure). On these grounds, each of the six sessions aimed for a 10 minute consultation. In reality each session tended to slide a scale; certain participant’s required shorter contact and others required slightly more. Sessions were spaced to optimise support and guidance, and protocol adherence. Phone calls were interspersed at 25-weeks with an outpatient clinic visit, to further reinforce the trials merits. Each call provided an opportunity for participants to raise any questions or concerns. An Excel® telephone tracker sheet was created to log each consultation date and provided a projected calendar of future contact for each person. In addition, paper tracking was constructed with the following details:

- Patient name, dietary group and telephone number/s. Columns for telephone contact dates (calls 1-6) and re-call requirements
- A concise cue point sheet to prompt the investigator before patient contact
3.8.3. Mobile Phone Text Reminders:

Short message service (SMS), or texting, was utilised in this study as a reminder for participants to fast before clinic arrival. A text message was sent to each individual on the day previous to their out-patient clinic visit. The request was no food to be eaten after midnight, and whilst water was permissible, other beverages such as tea and coffee were not. With regards to patient medication, as most patients were arriving for usual clinic consultation, they were reminded to follow general transplant outpatient protocol.

3.9. Week 25 data collection

In conjunction with week 25 data collection from both intervention groups, additional data was obtained from the records of non-intervention heart ($n=20$) and lung ($n=23$) transplant recipients. These individuals had met our inclusion criteria yet declined study participation. Data was collected for age, height and weight at baseline and 25 weeks. BMI was calculated at both time points (see Section 3.3.1). This data determined weight and BMI change in comparative non-intervention groups at the aforementioned time points only. Outpatient weight records have only recently (late 2015) become more routine.

3.10. Compliance Measures:

3.10.1 Food Frequency Questionnaire

The food frequency questionnaire (FFQ) used in this study was adapted from the US Nurses’ Health Study FFQ in the 1990s and validated for use among Australian adults [205]. The FFQ has since been updated to reflect the changing diets of Australians and has been found to be reproducible [206]. Further updates were incorporated by Wallingford et al [207] to reflect UK dietary choices, with subsequent minor changes applied for this study. The questionnaire was administered at baseline and 12-month visits. All data was entered into Excel® in duplication with the aid of a research assistant to minimise error.

3.10.2. Food Adherence Questionnaires

Participants were provided with either a low-fat or Mediterranean-based food adherence questionnaire (FAQ) extracted from the PREDIMED cardiovascular study [9], and minor translational corrections made (see Appendix). The low-fat FAQ contained 9 specific tick-box questions corresponding to fat-rich foods. The maximum score of 16 was based on a
given volume or abstinence of specific foods/groups and ranged from low (2), medium (1) or high (0) for questions 1, 3-4 and 6-9. Questions 2 and 5 are yes/no answers rated by a respective 1 or 0 point score. Conversely, the MeDiet FAQ incorporated 14 key food groups that are commonly consumed in the Mediterranean basin. A specific food volume or portion size denoted consumption frequency with a tick only applied to a positive answer. Both FAQs were conducted at baseline, 6 and 12-months. Participants were mailed an additional questionnaire at week-58 to help determine short-term post-study adherence. Ethical approval was granted for the additional FAQ time-point.

3.10.3. Questionnaire Validation

Validation was conducted to measure if data entered into FFQ and MeDiet and low-fat FAQs can adequately capture dietary habits in heart and lung transplant populations. UHSM outpatient heart (n=9) and lung (n=8) recipients assisted in conducting and returning the three differing questionnaires. To minimise bias, patients completed each questionnaire on separate days and in the following order:

- Day-1: FFQ
- Day-2: MeDiet FAQ (MD14)
- Day-3: Low-fat FAQ (LF9)

As discussed in section 3.10.1 the FFQ was previously validated in an Australian population. To ensure the food list reflected the UK diet, revisions were made, included cooking methods and specific types of oil, margarine, butter, cereals and takeaway foods. Additional vegetables were also included. Consumption frequency of each FFQ food item was converted to ‘servings per day’ intake by multiplying the standard serving size of each food (as specified in the FFQ) by the following values for each frequency option: Never=0; < once/month=0.02; 1–3 times/month=0.07; once/week=0.14; 2–4 times/week=0.43; 5–6 times/week=0.79; once/day=1.0; 2–3 times/day=2.5; and ≥4 times/day=4.0. Depending on the question items, daily servings (food/food group) relating to individual questions in MeDiet or low-fat FAQs were calculated by summing foods in each food group as ‘per day’ or ‘per week’.

Data from each FFQ and FAQ were compared. Final FAQ (MeDiet and low-fat) scores were also compared to the FFQ derived score.
Validation of the 14 and 9-point FAQs and comprehensive FFQ were conducted by at the Queensland Institute of Medical Research (QIMR) by an independent researcher. One method does not sufficiently describe bias; therefore a range of techniques were utilised for assessing agreement [208, 209].

To assess the agreement in each component of the FAQs and FFQ (i.e. categorical data), Kappa statistics were used [210, 211]. Interpretation of the Kappa coefficient was based on Landis and Koch’s classification: 0.00–0.20 = slight, 0.21–0.40 = fair, 0.41–0.60 = moderate, 0.61–0.80 = substantial and ≥0.81 = almost perfect agreement [212]. Since a highly skewed response to one category can substantially influence Kappa statistics [213, 214], crude agreement for each component of the MD14 and LF9 was presented. In addition, the Prevalence Adjusted Bias Adjusted Kappa (PABAK) statistics were also calculated to take into account the potential presence of both skewed responses and bias [209]. Expected agreement bias index were also calculated to facilitate the interpretation of PABAK [209].

The aforementioned independent QIMR researcher assessed the agreement of total scores from the two short screeners and the FFQ (i.e. quantitative measures), non-parametric Spearman rank correlation was used (data not normally distributed). Traditionally, correlation (either Pearson’s or Spearman) is calculated in the dietary intake validation studies; however, this approach does not display agreement and shows only the ‘correlation’ of measurements. Therefore, agreement of the two methods was further assessed by the limits of agreement (LA) [208, 215]. When assessing the agreement of different methods, a combination of Intraclass Correlation (ICC) and LA are recommended, as both approaches have inherited limitations [208]. Landis and Koch’s widely used classification enabled the ICC interpretation to be conducted [212].

3.11. Measurements of Intervention Acceptability

3.11.1. Household Member Feedback Questionnaire

An observation was made during wave one that a percentage of non-patients attending the training session reported health benefits. It was decided that a questionnaire be formulated to subjectively capture the opinion of individuals present at the training session, who would continue to occupy the same household as the study participant. A tick-box questionnaire was formulated with a five-point Likert scale and ‘additional’ comments section (see Figure G.) to assess opinion at study closure.
3.12. Sample Size

Since the AMEND-IT study was a pilot study it was not necessary or appropriate to perform power calculations to estimate sample size required to observe expected outcome. Over two years a combined total of 41 suitable candidates were identified and enrolled for both treatment arms. This number was the maximum number of patients that was logistically feasible for the single investigator to monitor and follow in the study period.

3.13. Clinico-biochemical Analysis

3.13.1. Immunoassay Detection:

Immunoassays are used routinely for on-site rapid and accurate detection of specific antibodies. An Abbott Architect c16000 Immunoassay Analyser situated at UHSM biochemistry department ensured accurate detected the following samples:

- Fasting glucose and insulin
- Lipid and steroid profile
- Urinary creatinine
- High-sensitivity c-reactive protein (hsCRP)

An Immunodiagnostics Systems IDS-iSYS™ automated system situated at Central Manchester University Hospitals NHS Trust provided analysis of the following sample:

- Insulin-like growth factor-1 (IGF-1)

Blood and urine samples collected at baseline, week 25 and 52 were collected and stored (see 3.7.2) for batch testing. Frozen samples were thawed and aliquoted into individual barcode detectable containers before testing. Both Architect and IDS-iSYS analysers were quality control checked before use. Results were obtained in mg/L, mmol/L, pmol/L or nmol/L and where applicable, corresponding figures were provided to calculate the incidence of sample haemolysis or lipaemia.

3.13.2. Haemoglobin Detection

Glycosylated haemoglobin A1c (HbA1c) was detected at baseline, week 25 and 52 using high-pressure liquid chromatography (HPLC). Blood was collected in an EDTA Vacutainer® (see section 3.7.1) and a 100µl whole blood sample was aliquoted, labelled and taken immediately to UHSM biochemistry department for analysis. HbA1c detection was
conducted using a calibrated and quality controlled Bio-Rad Variant™ II Turbo haemoglobin testing system. Results were reported with the newer IFCC (International Federation of Clinical Chemistry) standardised concentration units (mmol/mol).

3.13.3. Insulin Resistance

Insulin resistance, sensitivity and β-cell function were estimated at baseline, week 25 and 52 with the aid of a HOMA2 calculator developed by the University of Oxford diabetes trial unit. Fasting plasma glucose and insulin values were entered into the calculator to provide estimates of beta-cell function (%B) and insulin sensitivity (%IS) with a reading of 100% as normal, and insulin resistance (IR) with a normal value of 1.

3.13.4. Lipid calculations

The Friedewald Formula enabled LDL to be calculated with the following equation (total cholesterol, -HDL, -TG / 2.19). Fasting lipid measurements were used to further predict atherogenic risk by calculating ratios from cholesterol and TG measurements. Total cholesterol (Tchol) readings were divided by high-density lipoprotein to provide a Tchol/HDL ratio. The same method was used to calculate low-density lipoprotein and HDL (LDL/HDL) and TG and HDL (TG/HDL) ratios.

3.13.5. Urinary Polyphenol Detection

Fasting urinary samples were analysed (baseline only) using the total polyphenol quantification method highlighted in section 1.6.1. For this procedure, frozen urine samples were thawed on an ice bed, and then centrifuged at 4°C for 10 minutes at 3000rpm. 1ml of supernatant was removed from each sample, then diluted with 1ml of Milli-Q water and acidified with 34µL of 35% hydrochloric acid before loading into an Oasis® MAX solid-phase silica extraction cartridge. An extraction procedure was performed to filter the urine and 15µl of the eluted fractions were then added to a 96-well plate and mixed with 170µl of Milli-Q water. Standards were prepared from Sigma-Aldrich® analytical grade gallic acid and a serial dilution performed to represent the standard range. 12µl of Sigma-Aldrich® Folin-Ciocalteu’s (FC) phenol reagent was added to each test sample (and standard). FC reacts with phenolic substances to enable spectrophotometry detection. After 1 hour dark incubation, samples were read in a BioTek® Epoch Microplate spectrophotometer at an absorbance of 765nm.

3.14.1. Adherence data

Analysis for each diet group was performed in two sets. Firstly, estimated median change (Tables 2a and 2b) and median % change (Tables 3a and 3b) required a Wilcoxon signed rank sum test. The second sets of analysis were conducted with analysis of covariance (ANCOVA), a general linear model. This included baseline values as covariates and the follow-up scores as dependent variables. A p-value of <0.05 was considered statistically significant. Analysis of both food adherence and food frequency questionnaires was performed using SAS statistical software, version 9.4 (SAS Institute Inc.).

Statistical analysis of household member and feedback survey data was performed with SPSS software package, version 22.0. Data for this questionnaire was non-parametric; therefore a Mann-Whitney U test was performed. A p-value of <0.05 was considered statistically significant. In addition, analysis of the preliminary weight and BMI data at week 25 was also performed using the aforementioned Mann-Whitney U test.

3.14.2. Clinico-biochemical data

This study was designed around the primary question of ‘feasibility’. Emphasis was placed on generating sufficient data to power a larger Mediterranean dietary intervention study within a heart and lung transplant population. P-values were therefore not the specific focus of this study and will only be expressed where statistically relevant.

Analyses of anthropometric and biochemical data over the study period (incorporating baseline, 25 and 52 week data) were performed using a longitudinal Generalised Estimating Equation (GEE) regression model, with an autoregressive correlation structure, to account for the within-subject repeat readings using SPSS version 22.

The following between and within-subject differences were derived from the regression model:

1. Overall differences between diets (MeDiet/low-fat)
2. Overall differences between time points (Baseline, 25 and 52 weeks)
3. Differences in the pattern of change over time between the diet groups
Evidence of a significantly different effect of the two diets was shown by a significant difference in the pattern of change. Positively skewed data were log-transformed to obtain an adequate approximation to a normal distribution and geometric means are presented as suitable summary statistics for these data. Statistical significance was set at the conventional level of 5%.
Chapter 4: Results

Patient Characteristics, Feasibility and Acceptance

4.1. Introduction

A central precept of any dietary intervention is its implementation feasibility and overall acceptability. Therefore, the aim was to determine whether it was feasible to ask participants to change and maintain new eating habits and determine if they were willing to do so. Feasibility was indicated by uptake and drop-out rates and a comprehensive range of validated self-assessment questionnaires were administered to capture patients’ adherence and views, and also family members’ subjective responses.
4.1.1. Study flow-chart

![Study flow-chart diagram]

**Figure 2. Consort diagram of heart and lung recipient study involvement**

The investigator contacted 121 clinically stable, pre-screened heart and lung recipients (as described in Methods section 3.2.3), 69 (57%) individuals declined study participation and 11 other individuals expressed interest, but did not meet inclusion criteria (Figure 2). Reasons
for exclusion were diabetes \((n=6)\), low EGFR \((n=2)\), malignancy \((n=2)\) and organ rejection \((n=1)\). In total, 41 individuals (37% of those approached and eligible) consented and were randomly allocated to one of the dietary interventions. Two lung recipients exited the study at week 25 follow-up: one no longer wished to participate; the other died (chronic lung rejection). All remaining participants completed the study as scheduled at week 52.
4.1.2. Baseline Characteristics

| Baseline characteristics | Mediterranean diet  
\( n = 21 \) | Low-fat diet  
\( n = 20 \) | \( P \) value* |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>56.1 ± 7.8</td>
<td>54.3 ± 9.4</td>
<td>0.56</td>
</tr>
<tr>
<td>Male/female (( n ))</td>
<td>15/6</td>
<td>14/6</td>
<td>0.92</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.7 ± 15.3</td>
<td>81.9 ± 15.5</td>
<td>0.33</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>29.2 ± 4.3</td>
<td>28.62 ± 5.2</td>
<td>0.68</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>101.9 ± 11.9</td>
<td>100.2 ± 13.1</td>
<td>0.66</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>138.2 ± 12.8</td>
<td>141.3 ± 13.5</td>
<td>0.46</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>86.4 ± 10.8</td>
<td>88 ± 7.8</td>
<td>0.60</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>79.5 ± 12.5</td>
<td>79.4 ± 11.2</td>
<td>0.97</td>
</tr>
<tr>
<td>Imunosuppressive medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>14 (66%)</td>
<td>17 (85%)</td>
<td>-</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>7 (33%)</td>
<td>3 (15%)</td>
<td>-</td>
</tr>
<tr>
<td>Everolimus</td>
<td>1 (4.7%)</td>
<td>0 (0%)</td>
<td>-</td>
</tr>
<tr>
<td>Mycophenolate</td>
<td>13 (62%)</td>
<td>12 (60%)</td>
<td>-</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>5 (24%)</td>
<td>5 (25%)</td>
<td>-</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>21 (100%)</td>
<td>20 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Other medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthypertensive agents</td>
<td>16 (76%)</td>
<td>15 (75%)</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol &lt; medication</td>
<td>16 (76%)</td>
<td>16 (80%)</td>
<td>-</td>
</tr>
<tr>
<td>Heart transplant</td>
<td>10 (47%)</td>
<td>10 (50%)</td>
<td>-</td>
</tr>
<tr>
<td>Lung transplant</td>
<td>11 (52%)</td>
<td>10 (50%)</td>
<td>-</td>
</tr>
<tr>
<td>Time from transplant (days)</td>
<td>794</td>
<td>737</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Continuous values expressed as mean ± standard deviation
BMI = body mass index, BP = blood pressure
*Calculated with independent t test or Mann-Whitney U test according to data distribution after formal evaluation with Shapiro-Wilk test

Table 1 shows the baseline characteristics of heart and lung transplant recipients as randomised to each intervention. Baseline characteristics for both groups were homogeneous across all key parameters except that mean baseline weight was 4.8kg higher in the MeDiet cohort. Mean BMI values were however comparable. Total recruitment figures indicate the MeDiet group contained one additional participant at baseline.
4.2. Adherence

4.2.1. Mediterranean FAQ scores

Table 2. Mediterranean diet adherence: regular consumption of 14 specific food items/groups at four time points, based on a MeDiet screening questionnaire.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=21)</th>
<th>25 weeks (n=20)</th>
<th>52 weeks (n=20)</th>
<th>6-wks post-study (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVOO *</td>
<td>9 (43)</td>
<td>20 (100)</td>
<td>20 (100)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>EVOO (4tbsp&gt;/day)</td>
<td>1 (5)</td>
<td>14 (70)</td>
<td>14 (70)</td>
<td>13 (65)</td>
</tr>
<tr>
<td>Vegetable servings (2&gt;/day)</td>
<td>10 (48)</td>
<td>16 (80)</td>
<td>19 (95)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Fruit units</td>
<td>8 (38)</td>
<td>12 (60)</td>
<td>12 (60)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Red / Fat-rich meat</td>
<td>3 (14)</td>
<td>10 (50)</td>
<td>11 (55)</td>
<td>14 (70)</td>
</tr>
<tr>
<td>Dairy / vegetable fat</td>
<td>10 (48)</td>
<td>19 (95)</td>
<td>16 (80)</td>
<td>18 (90)</td>
</tr>
<tr>
<td>Sugar beverages</td>
<td>13 (62)</td>
<td>16 (80)</td>
<td>16 (80)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Red wine</td>
<td>1 (5)</td>
<td>5 (25)</td>
<td>4 (20)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Legumes</td>
<td>2 (10)</td>
<td>5 (25)</td>
<td>5 (25)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Fish / shellfish</td>
<td>8 (38)</td>
<td>16 (80)</td>
<td>13 (65)</td>
<td>13 (65)</td>
</tr>
<tr>
<td>Sweet baked goods</td>
<td>8 (38)</td>
<td>18 (90)</td>
<td>15 (75)</td>
<td>16 (80)</td>
</tr>
<tr>
<td>Nuts (unroasted)</td>
<td>3 (14)</td>
<td>10 (50)</td>
<td>8 (40)</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Lean meats ♠</td>
<td>15 (71)</td>
<td>19 (95)</td>
<td>17 (85)</td>
<td>17 (85)</td>
</tr>
<tr>
<td>Sofrito sauce †</td>
<td>4 (19)</td>
<td>15 (75)</td>
<td>12 (60)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Total score²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (min, max)</td>
<td>4 (1, 9)</td>
<td>10 (6, 14)</td>
<td>9 (5, 12)</td>
<td>10 (6, 14)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.4 ± 2.0</td>
<td>9.8 ± 2.1</td>
<td>9.1 ± 2.2</td>
<td>9.5 ± 2.1</td>
</tr>
</tbody>
</table>

Key: EVOO = extra-virgin olive oil
* = EVOO as main culinary oil
♠ = Poultry or lean game
† = tomato-based sauce with garlic, onions or leeks, simmered in EVOO
1 = No. of participants who answered ’Yes’ (score = 1)
2 = Total score ranged from 0 to14. Higher score indicates greater MeDiet adherence

Table 2 displays 14 food groups that formed the foundation of the traditional Mediterranean eating pattern that was recommended for those allocated to the MeDiet group. Scores represent the sum of MeDiet participants in waves 1 and 2 heart and lung groups to a maximum potential score of 14-points and according to the PREDIMED study upon which this questionnaire is based, adherence was defined as a total score of 9-points or greater, which represents 9 out of 14 MeDiet-defining food groups [9]. The median baseline score of 4 indicated participants were not following a Mediterranean eating pattern prior to allocation. A substantial change in food choice was observed at weeks 25 and 52, whereby total reported scores increased to 10 and 9, respectively. At week 58, 6 weeks after cessation of
intervention, total adherence scores were further maintained (median score 10). Extra-virgin oil and vegetable consumption scores increased significantly at all intervention time points, whilst dairy and vegetable fat intake decreased. Reported red and fatty meat intake also declined, whereas lean meat consumption increased slightly, intake was more consistent throughout the intervention period. In contrast, red wine and legume consumption remained consistently low over the 12-month period and raw nut intake remained moderately low.
### Table 3. Low-fat diet adherence: regular consumption of 9 specific food items/groups, based on low-fat screening questionnaire.

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n=20)</td>
</tr>
<tr>
<td><strong>Vegetable oil (tbsp./day)</strong></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>13 (65)</td>
</tr>
<tr>
<td>2-4</td>
<td>7 (35)</td>
</tr>
<tr>
<td>≥5</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Animal fat</strong></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Non-lean meat intake (servings/wk)</td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>5 (25)</td>
</tr>
<tr>
<td>2-4</td>
<td>13 (65)</td>
</tr>
<tr>
<td>≥5</td>
<td>2 (10)</td>
</tr>
<tr>
<td><strong>Dairy &amp; non-dairy fat (12g/wk serve)</strong></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>2 (10)</td>
</tr>
<tr>
<td>2-4</td>
<td>11 (55)</td>
</tr>
<tr>
<td>≥5</td>
<td>7 (35)</td>
</tr>
<tr>
<td><strong>Low-fat dairy</strong></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>10 (50)</td>
</tr>
<tr>
<td><strong>Fried foods (times/wk)</strong></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>15 (75)</td>
</tr>
<tr>
<td>2-4</td>
<td>4 (20)</td>
</tr>
<tr>
<td>≥5</td>
<td>1 (10)</td>
</tr>
<tr>
<td><strong>Fish/seafood * (times/wk)</strong></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>12 (60)</td>
</tr>
<tr>
<td>2-4</td>
<td>8 (40)</td>
</tr>
<tr>
<td>≥5</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Sweet baked goods † (servings/wk)</strong></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>7 (35)</td>
</tr>
<tr>
<td>2-4</td>
<td>7 (35)</td>
</tr>
<tr>
<td>≥5</td>
<td>6 (30)</td>
</tr>
<tr>
<td><strong>Nut/wheat crisps¥ (times/wk)</strong></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>6 (30)</td>
</tr>
<tr>
<td>2-4</td>
<td>10 (50)</td>
</tr>
<tr>
<td>≥5</td>
<td>4 (20)</td>
</tr>
<tr>
<td><strong>Total score</strong></td>
<td></td>
</tr>
<tr>
<td>Median (min, max)</td>
<td>10.5 (5, 14)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>10 ± 2.6</td>
</tr>
</tbody>
</table>

Key: tbsp. = tablespoon, wk = week
* canned in oil
* removal of visible animal fat
† commercial sweets or processed bakery products
¥ commercial wheat, corn, potato or nut snacks

Table 3 exhibits 9 specific foods (groups) that constitute the primary sources of dietary fat commonly encountered in the UK diet. Scores represent the sum of low-fat participants in...
wave 1 and 2 heart and lung groups. Unlike the MeDiet questionnaire, criteria for achieving a
distinct adherence score range were not set. Participant mean and median scores indicated
adherence increased for each food group at all intervention time points. However, these
changes were relatively modest when compared to reported baseline scores. Low-fat
participants reported substantial reductions in vegetable oil intake at week 25, 52 and 6-
weeks post-study, yet reductions in foods fried in oil were comparatively low throughout the
intervention (Table 3). Conversely, consumption of low-fat dairy products had increased at
week 25, but decreased again at 52 weeks, returning close to baseline levels at post-study
assessment ((Table 3). Dairy and non-dairy fat intake remained modest throughout the
intervention, whilst non-lean meat consumption is more evenly split between low and
moderate intake scores. Consumption of sweet-baked goods, nuts and crisps decreased
somewhat similar to the animal fat (excess removal) scores and consumption of fish and
seafood canned in oil.
4.2.3 Mediterranean adherence; combined FAQ and FFQ scores

Table 4. Mediterranean diet adherence based on regular consumption of 14 specific food items/groups extracted from MeDiet FAQ and FFQ.

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Baseline (n=21)</th>
<th>52 weeks (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVOO *</td>
<td>6 (29)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>EVOO (4tbsp&gt;/day)</td>
<td>9 (45)</td>
<td>19 (70)</td>
</tr>
<tr>
<td>Vegetable servings (2&gt;/day)</td>
<td>20 (95)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Fruit units</td>
<td>16 (76)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Red / Fat-rich meat products</td>
<td>13 (62)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Dairy / vegetable fat</td>
<td>12 (57)</td>
<td>17 (85)</td>
</tr>
<tr>
<td>Sugar beverages</td>
<td>13 (62)</td>
<td>16 (80)</td>
</tr>
<tr>
<td>Red wine</td>
<td>0 (0)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Legumes</td>
<td>8 (38)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Fish / shellfish</td>
<td>8 (38)</td>
<td>13 (65)</td>
</tr>
<tr>
<td>Sweet baked goods</td>
<td>13 (62)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Nuts (unroasted)</td>
<td>8 (38)</td>
<td>17 (85)</td>
</tr>
<tr>
<td>Lean meats ♠</td>
<td>1 (5)</td>
<td>11 (58)</td>
</tr>
<tr>
<td>Sofrito sauce †</td>
<td>8 (38)</td>
<td>16 (80)</td>
</tr>
<tr>
<td>Total score²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (min, max)</td>
<td>6 (3, 10)</td>
<td>11 (7, 14)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.4 ± 2.1</td>
<td>10.9 ± 1.7</td>
</tr>
</tbody>
</table>

Key: EVOO = extra-virgin olive oil
* = EVOO as main culinary oil
♣ = Poultry or lean game
† = tomato-based sauce with garlic, onions or leeks, simmered in EVOO
1 = No. of participants who answered ‘Yes’ (score = 1)
2 = Total score ranged from 0 to 14. Higher score indicates greater MeDiet adherence

The potential exists that participants may report the consumption of food items in the short adherence screener (FAQ) differently from the FFQ. Therefore, Table 4 represents reported baseline and 52 week MeDiet adherence scores (including % changes) that were correlated with corresponding FFQ food item data (please refer to Appendix D). All FFQs were completed solely at baseline and 52 weeks. The results reveal a positive increase in EVOO use, including total daily volume, which incidentally corresponds with greater EVOO-based sofrito sauce scores. Similarly, raw nuts, which are another MeDiet staple increased significantly. When cross tabulated, total vegetable consumption highlights an overall positive association, as participants were consuming two or more vegetable servings per day at baseline and 52 weeks. Conversely, fat-rich meat decreased, which coincided with an overall increase in lean poultry, fish and shellfish intake. Reductions in fat-rich dairy, vegetable oil and sweet-baked refined carbohydrate products are reported at the end of
intervention. However, red wine scores were zero at baseline as opposed to 3 participants at 52-weeks, indicating most patients abstained from consuming this Mediterranean beverage. Total median and mean scores demonstrate an overall dietary change at the end of study.
4.2.4. Low-fat diet: combined FAQ and FFQ scores

Table 5. Low-fat diet adherence: regular consumption of 9 specific food items/groups, based on FFQ data at baseline and end of intervention.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=20)</th>
<th>52 weeks (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable oil (tbsp./day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>6 (30)</td>
<td>5 (26)</td>
</tr>
<tr>
<td>2-4</td>
<td>8 (40)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>≥5</td>
<td>6 (30)</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Animal fat *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>9 (45)</td>
<td>11 (58)</td>
</tr>
<tr>
<td>Non-lean meat intake (servings/wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>6 (30)</td>
<td>10 (53)</td>
</tr>
<tr>
<td>2-4</td>
<td>7 (35)</td>
<td>4 (21)</td>
</tr>
<tr>
<td>≥5</td>
<td>7 (35)</td>
<td>5 (26)</td>
</tr>
<tr>
<td>Dairy &amp; non-dairy fat (12g/wk serve)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>5 (25)</td>
<td>12 (63)</td>
</tr>
<tr>
<td>2-4</td>
<td>9 (45)</td>
<td>7 (37)</td>
</tr>
<tr>
<td>≥5</td>
<td>6 (30)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Low-fat dairy 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>9 (45)</td>
<td>10 (53)</td>
</tr>
<tr>
<td>Fried foods (times/wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>7 (35)</td>
<td>10 (53)</td>
</tr>
<tr>
<td>2-4</td>
<td>5 (25)</td>
<td>4 (21)</td>
</tr>
<tr>
<td>≥5</td>
<td>8 (40)</td>
<td>5 (26)</td>
</tr>
<tr>
<td>Fish/seafood * (times/wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>6 (30)</td>
<td>3 (16)</td>
</tr>
<tr>
<td>2-4</td>
<td>8 (40)</td>
<td>9 (47)</td>
</tr>
<tr>
<td>≥5</td>
<td>6 (30)</td>
<td>7 (37)</td>
</tr>
<tr>
<td>Sweet baked goods † (servings/wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>6 (30)</td>
<td>12 (63)</td>
</tr>
<tr>
<td>2-4</td>
<td>8 (40)</td>
<td>5 (26)</td>
</tr>
<tr>
<td>≥5</td>
<td>6 (30)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Nut/wheat crisps ¥ (times/wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>6 (30)</td>
<td>4 (21)</td>
</tr>
<tr>
<td>2-4</td>
<td>8 (40)</td>
<td>11 (58)</td>
</tr>
<tr>
<td>≥5</td>
<td>6 (30)</td>
<td>4 (21)</td>
</tr>
<tr>
<td>Total score †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (min, max)</td>
<td>7.5 (1, 13)</td>
<td>9 (4, 16)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.7 ± 30</td>
<td>9.4 ± 2.8</td>
</tr>
</tbody>
</table>

Key: tbsp. = tablespoon, wk = week
* canned in oil
* removal of visible animal fat
† commercial sweets or processed bakery products
¥ commercial wheat, corn, potato or nut snacks
1 Low fat diet score ranged from 0 to 16. Higher score indicates greater dietary adherence
2 No. of participants who answered ‘Yes’ (score = 1)

Table 5 displays ‘raw’ low-fat baseline and 52 week adherence scores (including % changes) which correlate with corresponding food items reported in the FFQ scores. Data for week 25
and 58 is not provided as all FFQs were completed at baseline and 52 weeks only. When week 52 daily vegetable oil intake results are correlated with fat-based FFQ foods, it demonstrates total fat intake negatively increases in the ≥5tbsp/day bracket. This finding contrasts with the fried food scores, which signify a reduction in the number of fried meals consumed per week. Low-fat dairy intake positively increased, but not as substantially as the reported reduction in dairy and non-dairy fat. The latter findings were also similar for lean meat consumption and sweet baked goods. However, in regards to nut and crisps intake, the moderate score range of 2-4 increased at week 52 at the expense of reductions in high and low scores. Reductions in fat-rich meats occur, whereby the 0-1 serving per week bracket improves at the expense of high and moderate scores. Fish and seafood intake remained moderate overall, but unlike the FAQ, the FFQ data does not make a clear distinction between fish canned in oil or naturally oil-rich fish. Total median and mean scores demonstrate improved dietary change at the end of intervention.
4.2.5. Mediterranean FAQ scores: transplant type

**Table 6. MD14 scores**\(^1\) at each time point and score differences from baseline to each time point by transplant organ type using MD14 short questionnaire

<table>
<thead>
<tr>
<th></th>
<th>MD14 score</th>
<th>Differences from baseline</th>
<th>(p)-value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Mean±SD</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>(min, max)</td>
<td></td>
<td>(min, max)</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=21)</td>
<td>4.0 (1, 9)</td>
<td>4.4±2.0</td>
<td>—</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>4.0 (3, 7)</td>
<td>4.6±1.4</td>
<td>—</td>
</tr>
<tr>
<td>Lung (n=11)</td>
<td>4.0 (1, 9)</td>
<td>4.2±2.4</td>
<td>—</td>
</tr>
<tr>
<td><strong>25 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=20)</td>
<td>10 (6, 14)</td>
<td>9.8±2.1</td>
<td>5.0 (3, 10)</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>10 (7, 14)</td>
<td>10.2±2.2</td>
<td>5.0 (3, 10)</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>10 (6, 12)</td>
<td>9.3±1.9</td>
<td>5.0 (3, 7)</td>
</tr>
<tr>
<td><strong>52 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=20)</td>
<td>9.0 (5, 12)</td>
<td>9.1±2.2</td>
<td>4.0 (2, 8)</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>10.5 (7, 12)</td>
<td>9.8±2.1</td>
<td>5.0 (2, 8)</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>8.5 (5, 12)</td>
<td>8.4±2.1</td>
<td>4.0 (2, 6)</td>
</tr>
<tr>
<td><strong>6 wks post intervention</strong>(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=20)</td>
<td>10.0 (6, 14)</td>
<td>9.5±2.1</td>
<td>5.0 (–1, 10)</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>10.0 (7, 14)</td>
<td>9.8±2.4</td>
<td>4.5 (2, 10)</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>9.5 (6, 11)</td>
<td>9.1±1.8</td>
<td>5.0 (–1, 8)</td>
</tr>
</tbody>
</table>

\(^1\) MD score ranged from 0 to 14. Higher score indicates greater MeDiet adherence

\(^2\) \(p\)-values derived from Wilcoxon signed rank sum test

\(^3\) 58 weeks from baseline

Data in Table 6 represents total ‘raw’ MeDiet adherence scores from combined (and individual) transplant types, when observed at all intervention and post-study time points. Reported baseline scores reveal pre-intervention adherence to a Mediterranean-themed diet is particularly low. However, following protocol implementation an increased (consistent) adherence trend is observed at week 25, 52, and 6-weeks post-intervention. At week 25, heart and lung transplant recipients reported total mean scores of 10.2 vs. 9.3. Assessment at 52-weeks produces respective scores of 9.8 vs. 8.4, and this trend similarly continues at short-term post-study evaluation with respective scores of 9.8 vs. 9.1. Therefore, both MeDiet transplant groups significantly (\(p\)-value of <0.05 in all groups at all time points) increase reported adherence from baseline, with heart recipients reporting slightly greater adherence.
4.2.6. Low-fat FAQ scores: transplant type

Table 7. Low-fat scores\(^1\) at each time point and the differences from baseline by transplant organ type

<table>
<thead>
<tr>
<th></th>
<th>Low fat score</th>
<th>Difference from baseline</th>
<th>(p)-value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>Mean±SD</td>
<td>Median (min, max)</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=20)</td>
<td>10.5 (5, 14)</td>
<td>10.0±2.6</td>
<td>—</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>11 (5, 14)</td>
<td>10.3±2.9</td>
<td>—</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>10 (6, 13)</td>
<td>9.7±2.4</td>
<td>—</td>
</tr>
<tr>
<td><strong>25 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=20)</td>
<td>12 (9, 16)</td>
<td>12.4±1.6</td>
<td>2 (–2, 6)</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>13 (11,16)</td>
<td>13.0±1.6</td>
<td>3 (–1, 6)</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>12 (9, 14)</td>
<td>11.8±1.4</td>
<td>2 (–2, 6)</td>
</tr>
<tr>
<td><strong>52 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=19)</td>
<td>13 (8, 16)</td>
<td>12.7±2.2</td>
<td>2 (–2,10)</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>13 (12,15)</td>
<td>13.4±1.4</td>
<td>3 (–2, 7)</td>
</tr>
<tr>
<td>Lung (n=9)</td>
<td>13 (8, 16)</td>
<td>12.0±2.7</td>
<td>2 (–1,10)</td>
</tr>
<tr>
<td><strong>6 wks post intervention(^3)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=19)</td>
<td>12 (8, 15)</td>
<td>12.1±1.9</td>
<td>2 (–4, 9)</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>13 (10,15)</td>
<td>12.8±1.6</td>
<td>3 (–2, 7)</td>
</tr>
<tr>
<td>Lung (n=9)</td>
<td>11 (8, 15)</td>
<td>11.3±2.0</td>
<td>1 (–4, 9)</td>
</tr>
</tbody>
</table>

\(^1\) Low fat score ranged from 0 to 16. Higher score indicates greater dietary adherence
\(^2\) \(p\)-values derived from Wilcoxon signed rank sum test
\(^3\) 58 weeks from baseline

Table 7 displays ‘raw’ adherence scores collected from low-fat participants. With a \(p\)-value set at >0.05, results indicate statistical significance at all intervention time points. Baseline scores start higher in the low-fat group, resulting in smaller overall changes. Nevertheless, when stratified into heart and lung transplant groups, the reported mean scores calculated at week 25 (13 vs. 11.8) and 52 (13.4 vs. 12) indicate a slightly stronger adherence association in the low-fat HTx participants. Whilst significant positive change is maintained in the heart group at 6-weeks post-intervention, the LTx group fail to achieve a significant increase in adherence rating \((p=0.22)\) at this later time point. However, week 56 median and mean lung group raw scores still remained higher than baseline figures.
4.2.7. Mediterranean FAQ: percentage change

Table 8. Percentage differences from the baseline MD14 scores\(^1\) at each time point and stratified by transplant organ type\(^2\)

<table>
<thead>
<tr>
<th></th>
<th>Median (min, max)</th>
<th>Mean±SD</th>
<th>(p)-value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>25 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>35.7 (21.4, 71.4)</td>
<td>37.5±13.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>35.7 (21.4, 71.4)</td>
<td>40.0±16.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>35.7 (21.4, 50.0)</td>
<td>35.0±8.6</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>52 weeks</strong></td>
<td>28.6 (14.3, 57.1)</td>
<td>32.9±11.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>35.7 (14.3, 57.1)</td>
<td>37.1±14.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>28.6 (14.3, 42.9)</td>
<td>28.6±7.5</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>6 wks post-intervention(^3)</strong></td>
<td>35.7 (–7.14, 71.4)</td>
<td>35.4±17.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>32.1 (14.3, 71.4)</td>
<td>37.1±19.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>35.7 (–7.1, 57.1)</td>
<td>33.6±16.9</td>
<td>0.004</td>
</tr>
</tbody>
</table>

1 MD score ranged from 0 to 14. Higher score indicates greater MeDiet adherence
2 % difference is calculated as: \([(\text{scores at week 25 and 52, and post intervention}) – \text{baseline score}] / 14 \times 100\)
3 \(p\)-values derived from Wilcoxon signed rank sum test
3 58 weeks from baseline

Table 8 displays the food adherence questionnaire scores as a total percentage change from baseline. Median and mean scores at week 25 suggest a significant positive alteration in reported eating patterns when compared to baseline figures. Whilst this effect is statistically comparable in both heart and lung transplant MeDiet groups at week 52, the median and mean results decrease slightly in the lung cohort (35% vs. 28.6%). However, this overall positive trend continues in both transplantation groups at 6 weeks post-intervention demonstrating a strong, albeit reported, adherence association. When adherence scores for both groups are combined, a \(p\)-value of <0.001 is achieved at all intervention time points, and in the short term post-study. This indicates the acceptability of protocol implementation within these two transplant patient populations.
4.2.8. Low-fat FAQ: percentage change

Table 9. Percentage differences from low fat baseline scores\(^1\) calculated at each time point and stratified by transplant organ type\(^2\)

<table>
<thead>
<tr>
<th></th>
<th>Median (min, max)</th>
<th>Mean±SD</th>
<th>(p)-value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>25 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>12.5 (–12.5, 37.5)</td>
<td>15± 15.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>18.8 (–6.3, 37.5)</td>
<td>16.9± 17.9</td>
<td>0.027</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>12.5 (–12.5, 37.5)</td>
<td>13.1±13.0</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>52 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>12.5 (–12.5, 62.5)</td>
<td>17.1±19.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>18.8 (–12.5, 43.7)</td>
<td>19.4±19.2</td>
<td>0.020</td>
</tr>
<tr>
<td>Lung (n=9)</td>
<td>12.5 (–6.3, 62.5)</td>
<td>14.6± 20.7</td>
<td>0.043</td>
</tr>
<tr>
<td><strong>6 wk post intervention(^3)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>12.5 (–12.5, 56.3)</td>
<td>13.2±20.0</td>
<td>0.012</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>18.8 (–12.5, 43.8)</td>
<td>15.6± 18.5</td>
<td>0.039</td>
</tr>
<tr>
<td>Lung (n=9)</td>
<td>6.3 (–25.0, 56.3)</td>
<td>10.4± 22.5</td>
<td>0.22</td>
</tr>
</tbody>
</table>

\(^1\) Low fat score ranged from 0 to 16. Higher score indicates greater dietary adherence
\(^2\) % difference is calculated as: \([\text{score at weeks 25 and 52, and post-intervention} – \text{baseline score}] / 16 \times 100\)
\(^3\) \(p\)-values derived from Wilcoxon signed rank sum test

Table 9 displays comparative low-fat percentage change data from baseline in reported participant scores. The heart group maintain an improvement in low-fat eating habits at all intervention time points, a reported effect that continues with short-term post-intervention assessment. When the lung cohort is compared, their reported mean adherence scores also demonstrate a significant improvement in eating behaviour at 25 and 52 weeks. However, observations at 6-weeks post-intervention suggest this pattern regresses. The result is a non-significant \(p\)-value of 0.22; however, the percentage values provided at this time point remain higher than baseline. When adherence is calculated for both transplant types at this post-intervention time point, significance is strengthened by the increased statistical power \((p=0.012)\). Reported adherence is therefore only achieved in the heart group at the post-study time point.
4.2.9. Group comparison of FFQ derived scores

**Table 10. MeDiet\(^1\) and low-fat\(^2\) scores (minimum, maximum) derived from FFQ at two time points, including differences and transplant organ type**

<table>
<thead>
<tr>
<th>MeDiet scores</th>
<th>Median (min, max)</th>
<th>Mean±SD</th>
<th>52 weeks – baseline</th>
<th>p-value(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong> (n=21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>6 (4, 10)</td>
<td>6.7±2.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lung (n=11)</td>
<td>6 (3, 10)</td>
<td>6.1±2.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>52 weeks</strong> (n=20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>11 (7, 14)</td>
<td>10.9±1.7</td>
<td>5 (1, 8)</td>
<td>4.4±2.2</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>11 (7, 14)</td>
<td>10.9±2.2</td>
<td>5 (1, 8)</td>
<td>4.6±2.5</td>
</tr>
<tr>
<td><strong>Low-fat scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Baseline</strong> (n=20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>8.5 (4, 11)</td>
<td>8.1±2.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>6.5 (1, 13)</td>
<td>7.3±3.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>52 weeks</strong> (n=19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>10 (7, 14)</td>
<td>9±2.0</td>
<td>1.5 (–3, 8)</td>
<td>1.9±3.2</td>
</tr>
<tr>
<td>Lung (n=9)</td>
<td>8 (4, 16)</td>
<td>8.7±3.5</td>
<td>3 (–7, 7)</td>
<td>1.6±4.1</td>
</tr>
</tbody>
</table>

\(^1\) MD score ranged from 0 to 14. Higher score indicates greater MeDiet adherence

\(^2\) Low fat score ranged from 0 to 16. Higher score indicates greater dietary adherence

\(^3\) p-values derived from Wilcoxon signed rank sum test

Table 10 exhibits the differences between median and mean MeDiet and low-fat FAQ scores when compared against corresponding FFQ data. The table represents two distinct sets of data based on non-comparable FAQ scoring systems. In the MeDiet group, changes in comparative FAQ and FFQ data demonstrate a statistically robust change in eating habits for both transplant types at the end of intervention (week 52 \(p<0.001\)). Individual differences in MeDiet heart and lung adherence remained the same with positive improvements in both transplant types \((p=0.002)\). However, using the same comparative FAQ and FFQ data extraction criteria the low-fat intervention achieves a statistically significant change in eating habits at 52 weeks \((p=0.034)\), albeit with a weaker adherence association. When stratified into heart and lung recipients, the low-fat cohort fail to achieve statistically significant score differences at week 52 when FAQ and FFQ food group data is compared.
4.2.10. Group comparison of FFQ derived percentage change

Table 11. Percentage change of MeDiet\(^{1a}\) and low-fat\(^{2a}\) scores at 52 weeks
derived from FFQ by transplant organ type

<table>
<thead>
<tr>
<th></th>
<th>% change(^{1b})</th>
<th>p-value(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td><strong>MeDiet (52 wks)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=20)</td>
<td>35.7 (7.1, 57.1)</td>
<td>31.4±15.6</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>35.7 (7.1, 50.0)</td>
<td>30.0±13.4</td>
</tr>
<tr>
<td>Lung (n=10)</td>
<td>35.7 (7.1, 57.1)</td>
<td>32.9±18.2</td>
</tr>
<tr>
<td><strong>Low-fat (52 wks)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (n=19)</td>
<td>12.5 (–43.8, 50.0)</td>
<td>10.9±22.1</td>
</tr>
<tr>
<td>Heart (n=10)</td>
<td>9.4 (–18.8, 50.0)</td>
<td>11.9±20.1</td>
</tr>
<tr>
<td>Lung (n=9)</td>
<td>18.8 (–43.8, 43.8)</td>
<td>9.7±25.4</td>
</tr>
</tbody>
</table>

\(^{1a}\) MD score ranged from 0 to 14. Higher score indicates greater MeDiet adherence

\(^{1b}\) % difference is calculated as: \([(\text{score at 52 weeks})–(\text{baseline score})]/14 \times 100

\(^{2a}\) Low fat score ranged from 0 to 16, higher score indicates greater dietary adherence

\(^{2b}\) % difference is calculated as: \([(\text{score at 12-mths})–(\text{baseline score})]/16 \times 100

\(^3\) p-values derived from Wilcoxon signed rank sum test

Table 11 displays median and mean percentage changes in both MeDiet and low-fat groups. FAQ results were compared against corresponding data extracted from baseline and 52-week FFQs. Similar to Table 10, the scores represent two distinct data sets based on different FAQ scoring methods, hence the contrasting percentage ranges. Reported adherence in both MeDiet questionnaires strongly correlates with increased compliance at week 52 (p<0.001). These differences are equally significant between heart (p=0.002) and lung (p=0.002) transplant types. In comparison, the low-fat group (heart and lung combined) still attain a significant positive change in dietary adherence, but this association at week 52 is weaker than the MeDiet group (p=0.034). However, the low-fat group produces a non-significant finding when stratified for organ type at the same time point. Comparison of low-fat percentage change values demonstrates the total lung participant median score is greater than the HTx findings; yet p-values are weaker with wider disparity between minimum and maximum results. This suggests participants in this cohort are split between those who strongly adhered and others who were much less compliant.
4.2.11. Mediterranean index score

The MeDiet food adherence questionnaire is based on an alternate question and point scoring system than the low-fat FAQ. Therefore, an index was constructed (Figure 3) to better demonstrate representative change between interventions; for comparison see low-fat version (Figure 4). The reader must be aware that results are based solely on reported FAQ findings and are not correlated with FFQ data. The MeDiet index ranges from 0 to 100 (original MeDiet raw score x 7.143) with $p$-values from ANCOVA. No statistical differences in mean indices exist between organ type ($p>0.05$). However, at week 25 and 52, both groups demonstrate a positive and statistically significant difference from baseline intervention ($p<0.001$). This effect is maintained at week 58 post-intervention ($p<0.001$) suggesting both transplant groups continued to maintain short-term adherence at follow up assessment ($p<0.001$). A more linear pattern is observed in the heart cohort; an effect that is stronger at all time points than the lung cohort. In comparison, the overall (reported) change from 25 to 58-weeks suggests participants continued to eat food groups that are representative of a MeDiet, despite only receiving one initial training session. Nonetheless, this index clearly highlights a stronger positive effect in the HTx cohort.

![Figure 3. Mean MeDiet index by organ type over 58-weeks of intervention](image)
4.2.12. Low-fat index score

Figure 4 complements figure 3 by providing a low-fat index to which the MeDiet version may be compared and contrasted. The low-fat diet index ranged from 0 to 100 (original low fat diet raw score x 6.25) with \( p \)-values from ANCOVA. No statistical differences in mean indices exist between organ type \( (p >0.05) \). However, both groups demonstrate a statistically significant difference in overall reported adherence scores during the intervention. Baseline low-fat scores (~62) are higher than the MeDiet (~31) group (see Table 3a) suggesting food components of the low-fat FAQ were eaten more before intervention. Scores provided by both groups are rather linear, yet similar to the MeDiet index, this version also demonstrates a stronger adherence association in the HTx group at all time points. Furthermore, this observation continues at week 58 post-study assessment, and despite these differences, both heart and lung groups produce a statistically significant outcome \( (p<0.001) \) at this later time point.

Figure 4. Mean low-fat diet index\(^{1}\) by organ type over 58-weeks

<table>
<thead>
<tr>
<th></th>
<th>LF index</th>
<th></th>
<th></th>
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<td>75.2</td>
<td>70.8</td>
<td></td>
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<tr>
<td>6-mths</td>
<td>74.1</td>
<td>80.8</td>
<td>83.5</td>
<td>80.0</td>
<td></td>
<td></td>
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<tr>
<td>12-mths</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Post-intervention</td>
<td>70.8</td>
<td>80.0</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

\( p<0.001 \) (organ \( p=0.13 \)) \( p<0.001 \) (organ \( p=0.20 \)) \( p<0.001 \) (organ \( p=0.11 \))
4.3. Assessment of Eating Behaviours

4.3.1. Training Course Feedback Survey

Figure 5. Training day feedback survey: study participant anonymous scores after attending the training and cooking tuition class

Key: Questions 1-9
Q1. Prior to the day, how well informed did you feel?
Q2. Was background study information presented in an understandable manner?
Q3. Did (investigator) make good use of their time?
Q4. Was the chef’s cooking demonstration presented in a useful manner?
Q5. Did (chef) make good use of his time?
Q6. Has this session helped change your understanding of food for the better?
Q7. Was the printed information you received sufficient to keep you on track?
Q8. Was this event what you expected?
Q9. Overall, how satisfied were you with this event?
Figure 5 displays the individual and combined scores obtained from questionnaires sent to Mediterranean and low-fat training day participants \((n=19)\); 11 surveys were returned for wave-1 and 8 for wave-2. The questionnaires submitted do not represent the total number of participants; not all individuals returned the survey. The scores provided by both waves demonstrate study participants in both groups responded favourably to the structure and content of the training sessions. The survey was designed to be anonymous, therefore minimising reported bias or a perceived obligation to answer favourably. Findings in both groups were comparable for questions 1, 5, 6 and 7, with all other questions answered more positively. Question 1 relating to prior understanding of the study was answered slightly less favourably than the others questions, but still suggests a positive score.
4.3.2. Household member questionnaire

Figure 6 exhibits the mean distribution of household member questionnaire data collected from both Mediterranean \((n=13)\) and low-fat \((n=16)\) intervention groups. Similar to figure 5, the questionnaires submitted do not represent the total number of participants. Household members of both dietary interventions report a modestly improving trend for questions 1-5, but questions 3 and 4 are answered more weakly by MeDiet respondents. Question 6 suggests household members found shopping for new food items slightly more difficult, an effect that is interestingly more pronounced in the low-fat group. In addition, question 7 indicates these same household occupants in the MeDiet group observed no change in transplant recipient health, whilst the low-fat respondents provided a slightly negative score.
4.3.3. Discussion

This study identified that it is feasible to implement both Mediterranean and low-fat dietary education programmes in a heart and lung transplant outpatient population. Our novel findings are preceded by only one specific MeDiet observation reported in a post-heart transplant population and based on hypercholesterolemia outcomes [79]. Similar studies are absent for lung recipients, with further shortages for low-fat diet interventions in heart and lung transplant populations alike, further justifying the requirement for updated dietary assessment in these two patient populations. Of the 121 participants screened for eligibility, 42.9% expressed interest, of which a further 9.1% did not meet inclusion criteria. Overall, participant characteristics were equally balanced between interventions with the only notable difference observed in baseline weight measurements. Sex ratio in both groups was evenly matched; however the male preponderance in this study is similarly represented in the total current UHSM male (n=320) and female (n=125) patient listings.

Attrition rates are a key determinant of intervention feasibility; in this prospective study retention rates in both interventions were exceptionally high. The MeDiet cohort commenced with 21 participants, with a total of 20 completing the programme. In the low-fat cohort, 20 participants commenced but unfortunately one individual in wave-1 died of chronic lung rejection; an event not attributable to this study. The favourable attrition rates correspond with other MeDiet research findings that demonstrate compliance is achievable with adequate instruction and support [216]. In contrast, low-fat programmes generally elicit lower success rates [217]. This may be partly explained by a reduction in lipid-derived calories, which modulate appetite regulating hormones and delay gastric emptying to induce satiety [218, 219]. In addition, research also suggests that a low carbohydrate, higher fat/protein intake has a more substantial effect on subduing appetite [220]. Nevertheless, the low attrition rates in this small-scale prospective study were most likely influenced by a structured, personable approach. Moreover, the decision not to restrict energy intake was made early in the study as long-term self-monitoring is often difficult to consistently maintain, especially given the daily medication burden transplant recipients incur. Unlike the PREDIMED low-fat ‘control’ group, dietary fat intake in this study was not ascertained with a percentage figure prior to intervention. Our advice was similar, if not exceeding the fat reduction guidelines provided in this comparative study. The PREDIMED control group achieved a fat intake of 37% of total energy, which is well in excess of low-fat parameters. Furthermore, the PREDIMED trial reported a MeDiet dropout rate of 4.9%, and 11.3% in the control group [9]. This effect is
comparable to our MeDiet outcome; however the PREDIMED control group reported higher attrition rates, which would be expected considering the significantly greater sample size.

Acceptability measures centred on the use of two subjective self-administered questionnaires; one generalised and two intervention-specific versions. Validation concluded general compatibility of FAQ and FFQ methods, and importantly, respondent user-friendliness. In order to make further distinctions the investigator explored the use of an objective urinary biomarker. Unfortunately, detection of analytical standards proved problematic with subsequent urinary polyphenol validation requiring further evaluation. This limitation is highlighted in chapter 5.3. However, the use of two dietary questionnaires enabled robust data analysis to be performed. Combining key food components with a concise FAQ enabled simplicity of response and practitioner interpretation during consultation follow-up.

As previously mentioned, dietary lipids may contribute to satiety and two of the key foods attributed to a traditional Mediterranean eating pattern are EVOO and nuts. The 14-point FAQ helped determine the MeDiet groups’ adherence to these high caloric foods. Our results suggest nut intake was lower than the nut-supplemented PREDIMED group, who also determined nut intake with a combined questionnaire approach [221]. In this study, reported raw nut intake increased moderately from baseline in the 14-point FAQ, yet the combined FFQ calculations suggest overall intake was significantly higher. Unlike the PREDIMED study, nuts were not supplemented. MeDiet participants in this study were however supplemented with EVOO for the first 3-months, which may partly explain the significant increase in consumption at week 25. 100% of participants reported using EVOO at 12-months, whereas 70% of individuals reported achieving their daily target of 4tbsp per day at this same time point. This suggests regular EVOO use was maintained, even at post-study assessment. Baseline results for total raw MeDiet FAQ scores were low (4.4), but increased significantly at week 25 (9.8) and were maintained at week 52 (9.1) and post-study assessment (9.5). Conversely, the PREDIMED study (MeDiet) baseline adherence scores were greater than this current study [9]. When our MeDiet FAQ and FFQ scores are correlated, it reveals adherence at 52 weeks was more comparable to the PREDIMED findings.

As discussed in section 3.10.2, MeDiet and low-fat FAQs were based on two mutually exclusive formats, with separate scoring systems. Initial observation suggests greater adherence in the low-fat cohort, but each FAQ presents its questions differently. Unlike the low-fat FAQ, the MeDiet version asks participants about sugared beverages, red wine,
legumes and sofrito sauce. Whilst this does not affect overall adherence outcomes in each group, it makes between-group comparisons slightly more challenging. In order to strengthen conclusions, an index was created for total mean raw scores (combined organ type) at all time points. Although index scores were not directly comparable, the MeDiet findings at both follow-up time points achieved a much greater degree of change from baseline than the low-fat group. Moreover, the overall change in low-fat scores (52 weeks) is slightly weaker when the combined raw FAQ and FFQ data is observed. This effect was increasingly marked in the lung cohort at week 52 assessment. The MeDiet intervention demonstrates a positively stronger adherence association and this finding is consistent between both organ types at all measurements. Reported post-intervention eating pattern scores indicate that both MeDiet organ types maintain adherence at 58 weeks. In contrast, outcomes in the low-fat group highlight combined changes are significant, but when stratified into organ type, only the isolated heart cohort manages to achieve a significant difference.

Both groups were encouraged to consume similar foundational foods such as vegetables, fruit and wholegrains; therefore in essence the two interventions diverged primarily from a fat perspective. Comparative analysis of raw FAQ scores indicates vegetable oil intake significantly decreases in the low-fat group, whereas EVOO increases. This observation, whilst still statistically significant, is less robust in the combined low-fat FAQ and FFQ analysis. Furthermore, a similar trend occurs with animal and diary-based fat reduction, which again suggests greater overall adherence in the MeDiet cohort. Carbohydrate-rich foods contribute greatly to the prevalence of rising obesity rates [58], yet reductions in sweet-baked foods were more favourable in the MeDiet cohort at all points of assessment; a finding that corroborates with raw FAQ and combined FFQ calculations alike. Further isolated comparisons are more difficult to make due to divergence in FAQ construction.

Two further subjective measures provided a more holistic understanding of feasibility and adherence outcomes. The training seminar feedback form (see Appendix H) highlighted a consistent positive correlation across intervention waves, demonstrating favourable patient and household member opinion towards the education seminar structure. Reilly et al demonstrate in their qualitative study that family support was a key driver of positive dietary awareness and motivation [222].

In addition, household member opinion (see Appendix G) of individuals attending the training session was positive for both groups. Many of these individuals accompanied patients to outpatient appointments, which enabled further discussion at follow-up and
subsequent reinforcement of advice. A change in household member dietary choices offers reciprocal benefit as it may influence and support patient partner dietary decision making [223]. Shopping for new food items elicited no or slightly worse change and was moderately greater in the low-fat group. Considering this intervention group required the removal of specific fat-based items, whilst low-fat options such as dairy are widely available, this seems antithetical. Answers provided for patient health and wellbeing indicated ‘no change’, yet it must be borne in mind that interpretation of health-related quality of life changes is notoriously difficult. Estimation in chronic disease states must take into consideration ceiling effects, whereby room for improvement may actually be limited [224].

4.4. Anthropometric and Clinical Outcomes

4.4.1. Introduction

Above normal weight, waist circumference and BMI are considered primary indicators of overweight and obesity. Measurements taken at baseline, 25 and 52 weeks provide an easy and effective measure for determining basic physiologic changes after dietary intervention. In addition, hypertension is a recognised post-operative complication for heart and lung recipients though it is more commonly encountered in heart recipients, in part due to graft denervation and increased heart rate [149]. Blood pressure control often includes advice to follow a healthy diet, and both low-fat [225] and Mediterranean diets [21] can offer antihypertensive evidence-based approaches to ameliorate its effect. Hence these parameters along with other indicators of cardiovascular health such as heart rate were key primary outcomes of this pilot trial.

In the interests of the reader it is important to reinstate that as a feasibility study, the following clinico-biochemical results were not based on conclusive power calculations.
Table 12. Longitudinal analysis of anthropometric data

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>MeDiet Week 25</th>
<th>MeDiet Week 52</th>
<th>Baseline</th>
<th>Low-fat Week 25</th>
<th>Low-fat Week 52</th>
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<tr>
<td>Weight kg</td>
<td>86.2</td>
<td>84.1</td>
<td>84.4</td>
<td>82.0</td>
<td>82.0</td>
<td>81.8</td>
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<tr>
<td></td>
<td>(79.5, 92.8)</td>
<td>(77.9, 90.2)</td>
<td>(79.0, 89.8)</td>
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<td>(74.2, 89.5)</td>
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<tr>
<td>Time effect</td>
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<td>Diet group effect p=0.31</td>
<td>Diet group effect p=0.23</td>
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<td>Waist cm</td>
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<td>101.0</td>
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<tr>
<td>Time effect</td>
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<td>Diet group effect p=0.95</td>
<td>Diet group effect p=0.41</td>
<td>Diet group effect p=0.23</td>
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<td>BMI kg/m²</td>
<td>29.0</td>
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<td>(26.4, 30.8)</td>
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<td>(25.9, 31.2)</td>
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<tr>
<td>Time effect</td>
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<td>Diet group effect p=0.97</td>
<td>Diet group effect p=0.41</td>
<td>Diet group effect p=0.23</td>
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<td>BMR kcal/day</td>
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<td>1657</td>
<td>1652</td>
<td>1633</td>
<td>1632</td>
<td>1625</td>
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<td>(1520,1729)</td>
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<td>Diet group effect p=0.24</td>
<td>Diet group effect p=0.28</td>
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<td>Heart rate bpm</td>
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<td>Systolic BP mmHg</td>
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<td>143</td>
<td>140</td>
<td>141</td>
<td>138</td>
<td>137</td>
</tr>
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<td>(134, 145)</td>
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<td>(133, 146)</td>
<td>(135,147)</td>
<td>(132, 143)</td>
<td>(131, 143)</td>
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<td>87.6</td>
<td>88.0</td>
<td>89.4</td>
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Data calculated with GEE regression modelling and represented as mean values and 95% confidence intervals.
4.4.2. Weight assessment: week 25

Figure 7. Preliminary changes in body weight at baseline and 25 weeks

At week 25, wave-1 heart ($n=11$) and lung ($n=12$) recipient weight data were compared against 20 heart and 23 lung recipients that met our inclusion criteria, yet declined study participation. Comparative analysis of MeDiet and low-fat weight values (see Figure 7.) demonstrates no significant difference between each dietary intervention, in either $n=11$ heart ($p=0.773$) or $n=12$ lung ($p=0.261$) groups. However, when this respective data is compared against a ‘control’ group of evenly matched non-intervention heart and lung recipients, weight loss was statistically significant ($p=0.024$) in both dietary cohort (control group baseline characteristics not provided, see section 4.4.10). Moreover, median weight loss in the MeDiet and low-fat heart interventions was comparable, but similar findings in both dietary lung interventions were slightly weaker; an effect more marked in the low-fat group. Moreover, the LTx recipient ‘control’ group increased in weight as opposed to a loss incurred by the MeDiet and low-fat cohorts.
4.4.3. Weight

There was a slight decrease in mean weight in the MeDiet group after 52 weeks from 86.2kg to 84.4kg and no material change in weight in the low-fat group, but this difference was not significant (Figure 8).

Figure 8. Changes in body weight at baseline, 25 and 52 weeks
4.4.4. Waist Circumference

At 25 weeks both interventions exhibit a statistically significant reduction in waist measurement ($p=0.024$), but at 52 weeks the low-fat group slightly increase above baseline, whereas the MeDiet remain lower (Figure 9). Outcomes at this later time point were not significant.

Figure 9. Changes in waist circumference at baseline, 25 and 52 weeks
4.4.5. Body Mass Index assessment: week 25

Figure 10 represents preliminary BMI data calculated for both dietary interventions in heart ($n=11$) and ($n=12$) lung transplant recipients, when retrospectively compared against a non-intervention ‘control’ group of $n=20$ heart and $n=23$ lung recipients. Similar to the comparative weight data, these results indicate that comparative dietary intervention outcomes at week 25 were statistically non-significant in either heart ($p=0.804$) or lung ($p=0.292$) transplant populations. This finding differs however, when data is compared against an eligible control group that declined study participation (baseline characteristics not provided, see section 4.4.10), the data then reveals a significant comparative reduction in BMI in both Mediet and low-fat groups ($p=0.021$). Median BMI results in both dietary heart interventions represent a similar loss, yet the range of data provided by the MeDiet heart participant data is more tightly grouped. When contrasted with the lung recipient results, reductions in BMI are more robust in the MeDiet cohort producing a greater lower quartile reduction. Conversely, the low-fat findings in both transplant types share similar approximations.
4.4.6. Body mass index

**Figure 11. Changes in body mass index at baseline, 25 and 52 weeks**

Low-fat participant BMI remains linear throughout the study; conversely, the MeDiet cohort exhibits a -0.5 decrease at 52 weeks (Figure 11).
4.4.7. Basal Metabolic Rate

Figure 12. Changes in basal metabolic rate at baseline, 25 and 52 weeks

At 52 weeks a slight decrease in BMR was observed in both interventions and this effect was greater in the MeDiet group (Figure 12).
4.4.8. Heart Rate

A small mean decrease in heart rate from 79.4 to 76.9 was observed at 52 weeks within the low-fat intervention only, but virtually no change in the MeDiet group, and no difference between the interventions at 52 weeks (Figure 13).

Figure 13. Changes in heart rate at baseline, 25 and 52 weeks
4.4.9. Systolic and diastolic blood Pressure

Figure 14. Changes in systolic blood pressure at baseline, 25 and 52 weeks
Similarly systolic (-4mmHg) and diastolic (-3.5mmHg) blood pressure had both decreased at 52 weeks in the low-fat intervention group, but not the MeDiet group, which remained linear. These Findings occurred independently of any antihypertensive medication changes (Figures 14 and 15).

4.4.10. Discussion

Comparisons between week 25 MeDiet and low-fat weight and BMI measurements when calculated against non-intervention transplant recipients demonstrate positive findings in the intervention groups only. Whilst the comparative weight and BMI reduction at week-25 appears positive, it must be acknowledged that control baseline demographics are not provided in this body of work. Appropriate data would require retrospective ethical approval
and should be interpreted accordingly as characteristic variation could potentially bias outcomes. The non-significant weight and BMI findings at week 52 may appear counterintuitive to the positive dietary adherence results. Nevertheless, emphasis was placed on study feasibility and was therefore not powered. One explanation may be changes in muscle density and repair, as atrophy is often extensive during the pre, peri and early post-operative period. In some instances, fat loss at the expense of muscle gain must be born in mind as patients return to more normal routines. At the end of study a -1.8kg weight loss occurred in the MeDiet, compared to -0.2kg in the low-fat intervention. Comparative one year results from the PREDIMED study using participants identified with MetS features, indicates a mean weight increase of 0.3kg in their MeDiet+EVOO group and -0.1kg reduction in the low-fat control [226]. Given the characteristics of this group share many similar high CVD risk factors, and in light of increasing post-heart [227] and lung [43] transplant weight trends, our MeDiet findings may therefore be more pertinent.

The association between health risk and high waist circumference and BMI values is well documented in non-transplant populations [228]. The reduction in week 25 abdominal measurements occurred similarly in both interventions. Unlike the MeDiet group this did not coincide with a change in low-fat weight and this variation may be attributable to changes in activity levels, which were not ascertained. Nonetheless, at week 52 the low-fat group waist size increased by 0.8cm, compared to a -1cm reduction in the MeDiet intervention. The change in mean MeDiet waist circumference at 1 year is slightly greater than the -0.9cm average revealed in a MeDiet meta-analysis [31], and -0.55cm in the MeDiet+EVOO PREDIMED trial findings [229]. The PREDIMED low-fat control group fail to demonstrate a reduction in waist circumference, however they also did not achieve low-fat status as overall fat consumption (37% of total calories) was marginally less than the MeDiet intervention [9].

BMI assessment revealed a linear outcome in the low-fat intervention at all time points, contrasting with a slight MeDiet decrease at week 25 and 52. Significant BMI reduction (-1.8kg/m²) was demonstrated in one 12 week *ad libitum* MeDiet trial [230], but these findings are contrary to the Lyon Diet Heart study, which reported an increase in MeDiet BMI [78]. Conversely, Tuttle *et al* compared a low-fat versus MeDiet and concluded that BMI reduction was similar between groups (-1kg/m²) at 12 months [231]. Nonetheless, the limitations of BMI calculation were published in a meta-analysis and suggest abdominal obesity indices are more effective measures of CVD risk than BMI, due in part to more accurate quantification of abdominal fat distribution [232]. However, if as the literature suggests general post-
transplant trends correlate with >BMI and waist circumference, then the curtailed findings at 52 weeks may offer clinical relevance. This last point is substantiated by the 25 week preliminary ‘control’ group weight and BMI data (Figure 10). Unfortunately inconsistent outpatient weight records impaired the ability to conduct further assessment at week 52.

Resting energy expenditure (REE) was assessed using the predictive Harris-benedict (HB) equation. BMR values in the low-fat group remained static throughout the intervention, compared to the MeDiet group who appeared to decrease slightly at the end of study. However, reliability of the HB formula in heart and lung transplant recipients is problematic due to a lack of conclusive evidence. This equation has been shown to overestimate REE in non-transplanted patient populations, with accuracy significantly influenced by weight history [233]. Moreover, predictive equations were found to be far less accurate measures of REE than indirect calorimetry in a left-ventricular assist device population [234]. Given that fat-free mass (FFM) is the single greatest factor influencing BMR; observations must be viewed with caution. The average patient age in this study would suggest lean body mass (LBM) is in a declining phase. In addition, nutritional status is often compromised during the pre, peri and early post-operative period, which further contributes to deconditioning; an effect that may have been more marked in the wave-2 cohort. Overweight individuals exhibit lower REE due to increased fat mass at the expense of LBM, which may make predictive BMR calculations in this study prone to overestimation. Weight loss in non-exercising subjects is known to contribute to a loss in fat and FFM [235]. Unfortunately, exercise assessment was not conducted in this study making further interpretation difficult without a statistically significant effect.

The mean longitudinal changes between each dietary group reveal divergent patterns in BP and heart rate. Outcomes in the MeDiet group were characterised by a linear systolic and diastolic BP pattern, including heart rate, during the intervention period. A recent meta-analysis by Nissensohn et al, concluded a “positive and significant association between” the MeDiet and BP in non-transplanted individuals with mild hypertension. However, their intervention reported a small magnitude of effect in all cases [236]. The other important consideration in our high-risk patient population was that BP and heart rate were clinically well managed. However, we identify that no further increases occur in resting BP and heart rate over the intervention period, and any subsequent changes were not attributable to medication adjustments. The low-fat group revealed a slight decrease in BP and heart rate from baseline to 52-weeks. Elevated resting heart rate has been shown to negatively impact
long-term HTx survival [237], therefore a resting reduction of -2.5bpm in the low-fat intervention may confer clinical benefit. This is an important consideration given that hypertension following transplantation is often progressive and influenced by further independent risk factors such as cyclosporine medication [102]. 52 week reductions in systolic and diastolic BP were -4 and -3.5mmHg, respectively. Low-fat BP findings in other (non-transplant) studies such as the Dietary Approaches to Stop Hypertension (DASH) diet have previously described substantial decreases in systolic (-5.5mmHg) and diastolic (-3mmHg) BP [32]. It has been estimated that an 8% reduction in stroke and 5% decrease in CVD mortality could be achieved with a 3mmHg systolic reduction [238]. Whether this degree of effect can be extrapolated to heart and lung transplant recipients is not known. Considering that heart and lung transplant recipients were measured on aggregate using a small sample size, greater participant numbers would be necessary to explore further.

The 4-year blood pressure findings from the large PREDIMED trial also demonstrated that systolic and diastolic BP decreased significantly in both MeDiet and low-fat control groups. No systolic differences were observed between the low-fat control and MeDiet+EVOO interventions, yet diastolic outcomes were greater in both MeDiet cohorts. Their year-1 findings in all three intervention groups actually display mean systolic and diastolic figures less than those observed in our low-fat group [21]. One partial explanation may be that whilst dietary sodium reduction was discussed in our study, it was not specifically targeted with set amounts. Mean global sodium consumption exceeds the WHO recommendation of 2.0g per day in 88.3% of the world’s adult population with each daily reduction of 2.30g equating to a 3.82mmHg fall in systolic BP [239]. Braith and colleagues demonstrated that HTx recipients’ exhibit increased salt sensitivity [240], therefore a high or low intake may have influenced the outcomes in either of our diet groups. The ability to identify specific differences in total sodium intake in each intervention and transplant type was not determinable from the current FFQ. Given that BP commonly deteriorates following transplantation and is exacerbated with increasing age, strategies that help slow the progression of hypertension may offer patient benefit.
4.5. Clinico-biochemical Analysis

4.5.1. Introduction

The ability of heart and lung transplant recipients to maintain ‘normal’ glucose regulation is paramount as NODAT is a common occurrence. Glucose dysregulation is heavily influenced by immunosuppressive drugs; most notably tacrolimus, cyclosporine and prednisolone, and further complicated by elevated BMI and abdominal adiposity. Therefore, a comprehensive assessment of fasting glucose metabolism was conducted. Lipids not only provide an important energy source but also perform multiple structural and metabolic roles in human health. Nonetheless, impaired lipid metabolism may lead to chronic disease development in heart and lung recipients alike [241]. Therefore, a range of clinical measures were conducted to determine comparative changes when following a low-fat or fat-enriched Mediterranean diet. High-sensitivity CRP provides a more sensitive inflammation assay than standard CRP measurements. Its choice in this study aimed to detect subtle changes as plasma levels >3mg/L are independently associated with increased cardiovascular events compared to individuals with values <1mg/L [242].
Table 13. Longitudinal analysis of glucose regulation data

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>MeDiet</th>
<th>Low-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 25</td>
<td>Week 52</td>
<td>Baseline</td>
</tr>
<tr>
<td>Insulin pmol/L *</td>
<td>74.3</td>
<td>66.7</td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td>(60.6, 91.0)</td>
<td>(54.0, 82.4)</td>
<td>(58.2, 100.6)</td>
</tr>
<tr>
<td>Time effect p=0.38; Diet group effect p=0.64; Pattern of change over time between diet groups p=0.79</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Glucose mmol/L</td>
<td>5.45</td>
<td>5.52</td>
<td>5.30</td>
</tr>
<tr>
<td></td>
<td>(5.16, 5.75)</td>
<td>(5.08, 5.95)</td>
<td>(4.91, 5.68)</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c mmol/mol</td>
<td>39.2</td>
<td>39.0</td>
<td>37.6</td>
</tr>
<tr>
<td></td>
<td>(36.5, 41.9)</td>
<td>(35.9, 42.1)</td>
<td>(35.7, 39.4)</td>
</tr>
<tr>
<td>Time effect p=0.48; Diet group effect p=0.18; Pattern of change over time between diet groups p=0.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA2 IR *</td>
<td>1.41</td>
<td>1.26</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>(1.15, 1.72)</td>
<td>(1.02, 1.56)</td>
<td>(1.09, 1.77)</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>HOMA2 IS% *</td>
<td>71.2</td>
<td>79.1</td>
<td>80.6</td>
</tr>
<tr>
<td></td>
<td>(58.1, 87.2)</td>
<td>(64.0, 97.8)</td>
<td>(68.1, 95.4)</td>
</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA2 β% *</td>
<td>102.1</td>
<td>93.9</td>
<td>111.2</td>
</tr>
<tr>
<td></td>
<td>(88.3, 118.1)</td>
<td>(79.0, 111.6)</td>
<td>(89.4, 138.2)</td>
</tr>
<tr>
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<tr>
<td>IGF-1 ng/ml</td>
<td>222</td>
<td>202</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>(202, 242)</td>
<td>(182, 222)</td>
<td>(207, 277)</td>
</tr>
<tr>
<td>Time effect p&lt;0.001; Diet group effect p=0.46; Pattern of change over time between diet groups p=0.27</td>
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<td></td>
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</tr>
</tbody>
</table>

Data calculated with GEE regression modelling and represented as mean values and 95% confidence intervals
* geometric means derived from log transformation
IR = insulin resistance
IS% = insulin sensitivity
B% = beta-cell function
Table 14. Longitudinal analysis of lipid and high-sensitivity CRP data

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>MeDiet</th>
<th>Low-fat</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Week 25</td>
<td>Week 52</td>
<td>Week 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tchol mmol/L</td>
<td>5.79</td>
<td>5.33</td>
<td>5.43</td>
</tr>
<tr>
<td></td>
<td>(5.16, 6.43)</td>
<td>(4.92, 5.75)</td>
<td>(4.96, 5.90)</td>
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<td>MeDiet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.23</td>
<td>5.67</td>
<td>5.27</td>
</tr>
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<td></td>
<td>(4.85, 6.50)</td>
<td>(5.14, 6.20)</td>
<td>(4.75, 5.80)</td>
</tr>
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<td>p=0.80</td>
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<td></td>
<td>p=0.98</td>
<td></td>
</tr>
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<td>Pattern of change</td>
<td></td>
<td>p=0.80</td>
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<td>HDL mmol/L</td>
<td>1.49</td>
<td>1.48</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>(1.34, 1.65)</td>
<td>(1.32, 1.64)</td>
<td>(1.27, 1.64)</td>
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<tr>
<td>MeDiet</td>
<td></td>
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<tr>
<td></td>
<td>1.48</td>
<td>1.55</td>
<td>1.55</td>
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<td></td>
<td>(1.33, 1.63)</td>
<td>(1.36, 1.73)</td>
<td>(1.29, 1.80)</td>
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<tr>
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<td>Pattern of change</td>
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<td>4.01</td>
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<td></td>
<td>(3.54, 4.47)</td>
<td>(3.41, 4.08)</td>
<td>(3.27, 4.51)</td>
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<td></td>
<td>3.67</td>
<td>3.99</td>
<td>4.00</td>
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<td>(3.28, 4.07)</td>
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<td>(3.20, 4.80)</td>
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<td>Diet group effect</td>
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<td>Pattern of change</td>
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<tr>
<td>LDL mmol/L</td>
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<td>2.99</td>
</tr>
<tr>
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<td>(2.90, 3.91)</td>
<td>(2.68, 3.29)</td>
<td>(2.63, 3.35)</td>
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<tr>
<td>MeDiet</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>2.94</td>
<td>2.99</td>
<td>2.90</td>
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<td></td>
<td>(2.64, 3.24)</td>
<td>(2.59, 3.39)</td>
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<tr>
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<td>p=0.53</td>
<td></td>
</tr>
<tr>
<td>Pattern of change</td>
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<td>2.11</td>
<td>2.17</td>
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<td>(2.00, 2.73)</td>
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<td>(1.66, 2.68)</td>
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<tr>
<td>MeDiet</td>
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<tr>
<td></td>
<td>2.08</td>
<td>2.10</td>
<td>2.18</td>
</tr>
<tr>
<td></td>
<td>(1.80, 2.36)</td>
<td>(1.67, 2.53)</td>
<td>(1.66, 2.60)</td>
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<tr>
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<td></td>
<td>p=0.05</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Pattern of change</td>
<td></td>
<td>p=0.59</td>
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</tr>
<tr>
<td>Trigs mmol/L #</td>
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<td>1.77</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>(1.60, 2.13)</td>
<td>(1.50, 2.09)</td>
<td>(1.28, 2.16)</td>
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<tr>
<td>MeDiet</td>
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<tr>
<td></td>
<td>1.68</td>
<td>2.10</td>
<td>1.66</td>
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<td>(1.47, 1.93)</td>
<td>(1.64, 2.69)</td>
<td>(1.28, 2.13)</td>
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<tr>
<td>Diet group effect</td>
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</tr>
<tr>
<td>Pattern of change</td>
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<td>p=0.25</td>
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<td>Trig/HDL ratio #</td>
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<td>1.24</td>
<td>1.20</td>
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<td>(0.99, 1.54)</td>
<td>(0.83, 1.71)</td>
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<td>MeDiet</td>
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</tr>
<tr>
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<td>1.17</td>
<td>1.41</td>
<td>1.19</td>
</tr>
<tr>
<td></td>
<td>(0.95, 1.42)</td>
<td>(1.00, 1.99)</td>
<td>(0.87, 1.65)</td>
</tr>
<tr>
<td>Time effect</td>
<td>p=0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet group effect</td>
<td></td>
<td>p=0.87</td>
<td></td>
</tr>
<tr>
<td>Pattern of change</td>
<td></td>
<td>p=0.59</td>
<td></td>
</tr>
<tr>
<td>HsCRP mg/L #</td>
<td>1.48</td>
<td>1.51</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>(0.95, 2.29)</td>
<td>(0.83, 2.73)</td>
<td>(0.89, 2.50)</td>
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<tr>
<td>MeDiet</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1.90</td>
<td>1.00</td>
<td>1.08</td>
</tr>
<tr>
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<td>(1.03, 3.53)</td>
<td>(0.61, 1.64)</td>
<td>(0.89, 2.50)</td>
</tr>
<tr>
<td>Time effect</td>
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<td>Pattern of change</td>
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<td>p=0.94</td>
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</tbody>
</table>

Data calculated with GEE regression modelling and represented as mean values and 95% confidence intervals

*geometric means derived from log transformation
4.5.2. Fasting Insulin

Figure 16. Changes in fasting plasma insulin at baseline, 25 and 52 weeks

At 25 and 52 weeks, both the low-fat and MeDiet intervention groups display a decrease in fasting insulin values. Overall this effect was slightly more marked in the MeDiet group (Figure 16).
4.5.3. Glucose Regulation

Figure 17. Changes in fasting plasma glucose at baseline, 25 and 52 weeks

At week 25, fasting glucose readings decreased in both Low-fat and MeDiet groups (Figure 17). This effect continued in both dietary groups at week 52 and was statistically significant in both interventions when compared to baseline values ($p=0.03$).
4.5.4. Glycated Haemoglobin (HbA1c)

Figure 18. Changes in fasting HbA1c at baseline, 25 and 52 weeks

HbA1c decreased at week 25 and 52 in the low-fat group. However, the MeDiet intervention increases slightly (0.5mmol/mol) at 52 weeks (Figure 18).
4.5.5. Homeostatic Model Assessment-2: insulin resistance, sensitivity and beta-cell function

Figure 19. Insulin resistance estimated at baseline, 25 and 52 weeks using HOMA2 calculations
Figure 20. Insulin sensitivity estimated at baseline, 25 and 52 weeks using HOMA2 calculations
Insulin resistance, insulin sensitivity and β-cell function were estimated with HOMA2 modelling, and then the values log transformed to normalise data (Figures 19, 20 and 21). At week 25 insulin resistance decreases in both low-fat and MeDiet groups and continues across both interventions at week 52. Conversely, insulin sensitivity calculated at week 25 increases in both groups, but this effect is more pronounced in the MeDiet intervention. Sensitivity also increases at week 52, whereby the MeDiet group displays the greatest overall change. Moreover, β-function measured at 25 weeks increases slightly in the low-fat group, whereas the MeDiet group decrease. At 25 weeks the low-fat value decreases slightly but still remains higher than baseline. In contrast, the MeDiet group follow a similar trend yet remain closer to a 100% score at 52 weeks.

Figure 21. Estimated beta-cell function detected at baseline, 25 and 52 weeks using HOMA2 calculations
4.5.6. Insulin-Like Growth Factor-1 (IGF-1)

Figure 22. Changes in insulin-like growth factor-1, detected at baseline and 52 weeks

Fasting serum samples were screened for IGF-1 at baseline and 52-weeks only. Comparison of the low-fat baseline value reveals IGF-1 is higher than the MeDiet measurement, yet at 52 weeks the mean figures in both interventions are similar. This results in a decrease in the low-fat group of -37ng/ml, and -20ng/ml in the MeDiet intervention (Figure 22). The material change in both interventions was statistically significant at 52 weeks ($p<0.001$).
4.5.7. Total Cholesterol

At week 25, fasting total cholesterol (Tchol) decreased from baseline in the low-fat and MeDiet interventions. Both dietary groups continue this pattern at week 52, which resulted in comparable values (Figure 23). This outcome was statistically significant ($p=0.007$) and occurs independently of lipid lowering medication changes, which also applies to the other lipid outcomes observed in this study.

**Figure 23. Changes in total cholesterol at baseline, 25 and 52 weeks**
4.5.8. High Density Lipoprotein

Figure 24. Changes in high-density lipoprotein at baseline, 25 and 52 weeks

Analysis of fasting mean HDL at week 25 reveals the low-fat and MeDiet group readings were the same as baseline. At 52 weeks, the MeDiet values remain linear, whereas the low-fat intervention decreases slightly (Figure 24).
4.5.9. Total Cholesterol and HDL Ratio

Figure 25. Changes in total cholesterol and high-density lipoprotein at baseline, 25 and 52 weeks

Total cholesterol and HDL ratios determined at week 25 indicate a slight reduction in both low-fat and MeDiet groups. At week 52, this trend continues in the MeDiet group whilst the low-fat intervention returns to baseline values (Figure 25).
Comparison of baseline and week 25 LDL measurements indicates a decrease in the MeDiet group only, whereas the low-fat intervention measurements remain static. At 52 weeks the MeDiet exhibits a further decrease; in contrast the low-fat group also display a reading less than baseline (Figure 26).
4.5.11. LDL and HDL Ratio

LDL/HDL ratios were calculated at week 25 and result in a low-fat group increase above baseline, whereas the MeDiet group ratio decreases at this same time point (Figure 27). Conversely, at week 52 the low-fat group remains similar to baseline, but the pattern of change between each intervention reveals that participants in the MeDiet group exhibit a significantly lower LDL/HDL ratio at 52 weeks when compared to baseline ($p=0.05$).
4.5.12. Triglycerides

Figure 28. Changes in fasting TG levels at baseline, 25 and 52 weeks

Fasting triglyceride measurements required log transformation of data to normalise non-parametric values. At week 25 both interventions display a value less than baseline measurement, though this effect is more marked in the low-fat group. At 52 weeks, this trend continues to provide similar results in both diet groups (Figure 28). However, whilst the overall change from baseline was slightly greater in the low-fat group, the material change in both interventions was statistically significant at both time points ($p=0.004$).
4.5.13. Triglyceride and HDL Ratio

The TG/HDL ratios were calculated using log transformed data. At week 25, both the low-fat and MeDiet groups display a ratio reduction from baseline readings. This pattern continues at week 52 to produce a slightly greater rate of change in the low-fat intervention (Figure 29).
4.5.14. High-sensitivity C-reactive Protein

Data provided from hsCRP results reveals both interventions contained a significant number of outlying participants that coincide with clinical observations made independent of this dietary intervention study; therefore, values were log transformed to normalise data. At week 25, hsCRP increased slightly in both the low-fat and MeDiet group. This pattern continued at week 52 (Figure 30).

Figure 30. Changes in baseline, 25 and 52 week high-sensitivity C-reactive protein
4.5.15. Discussion

As discussed in section 1.5.2, dysglycemia increases significantly in post-operative heart [14] and lung [15] recipients alike. The findings from this prospective study indicated no significant change in fasting blood sugar regulation (glucose withstanding) for either dietary intervention. However, combined assessment of glucose metabolism reveals a more integrated picture. Insulin is sensitive to blood glucose changes, yet impairment as observed in type-2 diabetes (T2D) would be expected to coincide with hyperglycaemia. On the contrary, this effect is not observed as blood glucose decreases in both interventions at the end of study. In general populations a fasting blood glucose (FBG) reading of 5.6mmol/L or greater is accepted as the threshold for impaired glucose tolerance [243], yet our baseline values were below this parameter. A large single-centre study identified NODAT in 73% of kidney transplant recipients with a FBG reading of ≥5.6mmol/L [244]. In this study, mean FBG values for both groups decreased similarly at 52 weeks suggesting greater glycaemic control. An oral glucose tolerance test (OGTT) was not conducted in this analysis, but is considered the gold-standard when further identifying pre-diabetic patients [245].

Fasting insulin levels decreased at all time points in both low-fat and MeDiet groups, but if viewed in isolation, one could interpret the decreased insulin secretion as a progressive defect. However, obesity is linked to insulin resistance [246] and the average BMI defined participants in both brackets as overweight, but the lack of significant weight loss suggests decreased insulin readings in both interventions occurred independently of weight reduction. Healthy, lean populations have a fasting insulin range of 18-90pmol/L [247], in contrast fasting insulin readings from a large diverse patient population study demonstrated concentrations ranging from 50.2 to 97.7pmol/L [248]. Fasting insulin measurements within heart and lung transplant populations are sparse, but the aforementioned values suggest our participants were within acceptable range.

HOMA2 modelling determines normal β-cell function with an estimate of 100% in non-transplanted individuals [249]. Using the same criteria, an insulin resistance (IR) score of 1 is also viewed as normal. Specific heart and lung transplant studies are lacking but our data demonstrates β-cell function in the low-fat group was greater than 100% at baseline, 25 and 52 weeks, whereas the MeDiet group remain closer to 100% throughout the intervention. To adequately interpret changes in glucose regulation, β-cell function must be reported in tandem with insulin sensitivity [249]. Progressive β-cell failure and IR promote T2D development. IR scores in both groups were elevated above 1 at baseline but decrease in a
similar pattern throughout the intervention. Lower FBG and insulin measurements should reflect the significant reduction in dietary carbohydrate intake reported in the adherence scores. However, it should be noted that corticosteroid medication is associated with IR and decreased glucose utilisation [250]; therefore, tailored reductions in prednisolone dosing throughout the intervention may have contributed to lower IR readings. Within heart and lung transplantation, established HOMA2-IR reference values are currently lacking; however, Geloneze et al produced a HOMA2-IR cut-off range for metabolic syndrome and insulin resistance in a Brazilian population. Their findings identify a cut-off value of 1.8 for insulin resistance and 1.4 for metabolic syndrome. In comparison, our results identify a decrease at 52 weeks, producing low-fat and MeDiet scores of 1.29 and 1.24, respectively. Furthermore, hypertriglyceridemia has been linked to post-heart transplant insulin resistance [251], but analysis indicates fasting TG levels decreased towards ideal clinical range at the end of study. This further substantiates our positive adherence findings as lower plasma TG levels correspond with our advice to consume lower glycaemic foods.

However, if we compare IR estimates with the HOMA2 insulin sensitivity (%S) values, a greater prognostic pattern may be observed. HOMA2 takes 100% IS as normal functioning, and subjects in both diet groups are below this threshold at baseline (~70%), but increase similarly at both intervention time points. Wallace states that “the concept of the model [HOMA2] is that %S is a function of glucose metabolism driven by the action of insulin” [252]; therefore, a subsequent decrease in IR, and increase in %S, may imply better glucose regulation. Insulin sensitivity also shares a close relationship with adiposity; however, a lack of significant change in weight and waist measurements at the end of study suggests improved %S estimates occurred via other means.

The American Diabetes Association list a HbA1c reading of 38.8mmol/mol as a pre-diabetic risk factor (not clinical outcome), which is further potentiated with a FBG reading of 5.6-6.9mmol/L [243]. HbA1c values reflect blood glucose exposure over 8-12 weeks, and a reading higher than 42.1mmol/mol identified adults at risk of diabetes and CVD in a large US study, even after adjustment for fasting glucose [253]. Our findings reveal HbA1c decreased in the low-fat group by 1.6mmol/mol at the end of intervention, thus remaining within ‘non-diabetic’ status (25-36mmol/mol) according to current NHS guidelines. Conversely, the MeDiet group increased by 0.5mmol/mol, which using the same criteria characterises them with ‘good control’ (<49mmol/mol). These overall changes are small, but given no further increases occur at 52 weeks this may be a positive finding as glucose dysregulation follows a
progressive pathway in immunosuppressed transplant recipients. In spite of the lack of statistical significance, when glucose regulation results are viewed as an aggregate they suggest an improving clinical association.

IGF-1 is not a typical predictive marker of diabetes; nonetheless, low circulating IGF-1 levels have been shown by Sesti et al to be an independent predictor of reduced insulin sensitivity [14], yet in this study insulin sensitivity increased at the end of intervention. Moreover, current research by Grogan et al, demonstrates IGF-1 was not associated with type-2 diabetes mellitus risk (T2DM), but binding protein IGFBP-3 (not measured) was at higher levels [15]. Transplant-based IGF-1 measurements are currently limited, but a paper published by Bidlingmaier et al enabled identification of background averages. Their findings reveal our patient samples were within ‘normal’ range, but overall mean values were higher than age-matched comparators [254]. The pleiotropic action of IGF-1 is not easily defined in transplant and non-transplant populations alike, but increased IGF-1 has been linked to BOS incidence in LTx patients [16]. Conversely, serum IGF-1 levels in the range of 159.7±114 ng/mL have been reported in HTx recipients with CAV, compared with non-CAV patients (234.1±136 ng/mL), which suggests IGF-1 is an additional marker of cardiac performance [255]. Our findings illustrate that IGF-1 decreased to an approximate threshold of 200ng/ml in both interventions. Whether this change was attributable to nutritional changes or other factors is not known. A positive association exists between greater animal protein intake and increased serum IGF-1 in non-transplanted middle aged males. The values determined by Larsson et al, reveal baseline IGF-1 was significantly lower than the measurements observed in this current study. [256]. Given the similar age range of their participants, it suggests circulating IGF-1 levels are potentially higher in transplanted individuals. Nonetheless, elevated IGF-1 shares a relationship with increased cancer risk in general populations [257, 258], and in light of malignant changes occurring frequently in immunocompromised transplant recipients [259], decreases in both groups may be clinically relevant. Conversely, Levine and colleagues clearly demonstrate that dietary protein restriction reduces circulating IGF-1 concentrations. In their study, high animal protein intake (<65yrs of age) elevated serum IGF-1 and was strongly associated with cancer incidence and diabetes-related mortality over an 18 year follow-up period [260]. The authors of this large study do not differentiate between red meat and fish intake; yet reported red meat intake in our prospective study was significantly reduced over the intervention period, and may have contributed to decreasing IGF-1 trends. As previously mentioned, Levine et al note a correlation between greater fish consumption and increased IGF-1, but the reported fish intake in this study coincides with the opposite
effect. Dietary restriction is further associated with lower IGF-1 expression [261], but an overall lack of weight loss in both groups does not imply a significant caloric decrease that explains our findings.

In spite of universal lipid lowering medication, dyslipidaemia’s are a frequent occurrence post-transplant. The ability to determine combined changes in lipid patterns may be more clinically relevant, especially when outcomes occur independently of lipid lowering medication. At 52 weeks, Tchol in both low-fat and MeDiet groups decreased closer to the desired clinical range of ≤5mmol/L. Findings from the Lyon Diet Heart Study revealed that for each 1mmol/L rise in Tchol, the recurrent risk of cardiovascular complications increased by 18 to 28% [216]. The decrease in Tchol in this study did not exceed 5mmol/L, but was comparable between groups at the end of intervention. Considering the intake of EVOO and oily fish increased significantly in the MeDiet group, the overall decrease in Tchol is in keeping with observations from a recent MeDiet meta-analysis [262]. In addition, a 10% Tchol reduction was encountered in a MeDiet kidney transplant study; an outcome that is similar to our findings [263]. Conversely, a meta-analysis of the low-fat DASH diet reported a significant reduction in Tchol of 0.2mmol/L, which was less than the 0.4mmol/L observed in this study [264].

The MeDiet group also exhibited a reduction in Tchol/HDL ratio, unlike the low-fat intervention that remained similar to baseline at the end of study. It has been suggested in a large non-transplant population that a Tchol/HDL ratio greater than 4.5 infers significant CVD risk [265]. Using this reference value, both groups remained within appropriate target range throughout the intervention. Schaefer et al report an ad libitum low-fat diet produced no change in Tchol/HDL ratio in a moderate hypercholesterolemic group of middle aged patients. However, unlike our low-fat group, weight loss and LDL were significantly reduced [266]. A 3 month prospective PREDIMED study observed a similar static effect in their low-fat group, and a -0.17 MeDiet ratio reduction [267], compared to -0.34 in this present study. An alternative study comparing low-fat, low-carbohydrate and MeDiets concluded greater reduction in the MeDiet cohort compared to the low-fat group. However, the greatest inverse effect occurred in the low-carbohydrate intervention, which interestingly produced the most significant increase in HDL [268].

The Framingham study helped characterise the strong inverse association between low HDL and CVD [269]. Similarly, HDL has been shown to increase significantly with adherence to a MeDiet [262], but in this intervention no further change was observed from baseline in our
MeDiet group. Conversely, our low-fat group remained unchanged at week 25, but then decreased slightly at 52 weeks. This pattern has been described in a 3 month MeDiet and low-fat comparison trial, whereby HDL changes remained non-significant in both cohorts [270]. Overall, the literature suggests that a low-fat eating pattern is less effective in raising serum HDL [271-273]. A comprehensive meta-analysis of low and high fat diets further highlights the positive association between elevated HDL and high mono and polyunsaturated fat intake [50]. Considering that monounsaturated EVOO increased significantly in this study, it is interesting that our outcomes signify a linear effect in the MeDiet group. The same study also provides evidence that low (saturated) fat diets contribute to a significant decline in LDL. In this current study low-fat and MeDiet groups exhibited decreasing LDL values, and this may be explained by the findings of Violi et al who observed a post-prandial reduction in LDL and oxidised LDL (oxLDL) after administering EVOO [274]. The relationship between elevated LDL and CHD is well established [275], but research also implicates oxLDL in inducing vascular endothelial damage and subsequent atherogenesis [276]. The PREDIMED group report that a traditional MeDiet was more effective than the low-fat control in reducing oxLDL in a heart failure subgroup [277]. This is a positive outcome, yet it brings us back to the earlier point that their low-fat intervention failed to make substantial reductions in total fat intake. The decreased LDL trend in our low-fat intervention is indicative of greater adherence and substantiated by the positive adherence questionnaire findings. Delahoy et al state a -14% reduction in major vascular events and a -16% decline in coronary episodes can be achieved with a LDL decrease of -0.65mmol/L [275]. In contrast, our MeDiet outcome of -0.46mmol/L would then infer translational benefit. Despite this both interventions conclude with similar LDL values, which remain above NHS guidelines (2mmol/L) for high risk patients.

CVD risk can also be predicted by calculating LDL/HDL ratios, which may offer greater predictive merit than isolated lipoprotein values. A high ratio increases the disparity between atherogenic and protective components, an effect that becomes even more pronounced in combination with hypertriglyceridemia [140]. The Helsinki study provided evidence that a LDL/HDL ratio >5, in combination with a triglyceride reading >2.24mmol/L predicted the highest number of coronary complications [278]. Similar findings were reported in the PROCAM study, whereby coronary events occurred six times more frequently when LDL/HDL ratios exceeded 5mmol/L. Moreover, total risk also doubled with a triglyceride measurement higher than 2.24mmol/L [279]. These findings were encountered in patients within the mean age range of our participants and occurred twice as frequently in men, which
again were proportionately higher in this current study. LDL/HDL ratios in both our interventions were less than half those observed in the aforementioned studies. Furthermore, triglyceride outcomes remained well below 2mmol/L in both groups, with a significant reduction of 0.29mmol/L or 11.5% in the MeDiet group. This value is identical to that observed in a MeDiet study observing the cholesterol lowering potential of phytosterol-rich plant foods [280], whereas the low-fat intervention experienced no significant change.

The relationship between elevated TG and various atherogenic lipoproteins increases the residual risk of CVD even when LDL measurements are brought within target range using statin therapy. Hypertriglyceridemia presents a significant risk factor for future cardiovascular events and is frequently encountered in T2D and MetS patients [281]. Moreover, Cottini and colleagues demonstrate an association between elevated TG levels and primary graft dysfunction following LTx [241]; an effect that also correlates with organ rejection in HTx recipients [95]. Significant reductions in serum TG have been reported with greater adherence to a reduced carbohydrate diet [282, 283]. However, Vitale et al produced a more comprehensive analysis of dietary fat and carbohydrate intake in T2D patients. Their results demonstrate it is the quality of carbohydrate, subsequent glycaemic load and fibre component that are responsible for influencing serum triglycerides [284]. Our advice was based on unprocessed lower glycaemic foods, and the positive adherence results in both interventions corresponded with decreased TG levels that exceeded current CVD-risk guidelines (<1.7mmol/L) at the end of study. Nonetheless, a meta-analysis concluded that MeDiets induce significant beneficial effects on fasting TGs [285]. Both our interventions produced similar outcomes and it is hypothesised that the increased satiety of EVOO may have contributed to the MeDiet group consuming more vegetables at the expense of carbohydrate-based calories.

Decreasing TG trends were repeated in both interventions for TG/HDL ratios. This ratio provides a robust and predictive capacity for determining CVD and all-cause mortality risk in men [286] and women [287]. A ratio of <2 constitutes the desired target range in at risk populations, which suggests our baseline values were clinically well controlled. However, over the course of this intervention both dietary groups achieve TG/HDL ratios that occupy clinical ranges associated with lower adverse outcomes [288]. Biadi et al [289] and Raichlin and colleagues [290] both report that a TG/HDL ratio of >3 in combination with a CRP reading >3ml/L were significantly associated with CAV incidence. Inflammation influences every aspect of disease; therefore the ability to detect subtle subclinical changes can offer
profound diagnostic advantages. Persistent pro-inflammatory events have been discussed in response to hsCRP measurements $\geq 3$mg/L, which places patients at significantly increased cardiovascular risk, compared to individuals <1mg/L [291]. Notwithstanding, elevated CRP shares a direct association with both overweight and obesity [292] and increased waist circumference [293]. Mean BMI and waist measurements in both groups fall within central obesity parameters, which may have contributed to the slightly elevated values. However, CRP is a non-specific inflammatory biomarker produced in response to elevated interleukin-6 and initiated by tissue injury and infection. This may further explain the skewed data distribution as a selection of participants in both groups presented with clinical complications independent of this study, such as a gallbladder infection or chronic rejection.
Chapter 5: Overall Discussion

The following chapter will discuss the primary findings of this study and present these in the context of current knowledge and clinical and practical implications.

5.1. Summary of Main New Findings

The findings from this study provide evidence that the implementation of a comprehensive healthy eating programme is feasible in both heart and lung transplant outpatient populations. By chance due to the small total numbers of patients involved, the low-fat group commenced the study with higher baseline scores despite randomisation. However, comparative assessment of the Mediterranean and low-fat diet interventions reveals that whilst adherence to both interventions was high at the end of study, associations with beneficial outcomes were slightly stronger in the MeDiet group. Decreasing trends in weight, waist circumference and BMI occurred in the MeDiet group only. Nonetheless, when both diet groups were compared at week 25 against eligible non-intervention heart and lung recipients, a significant weight and BMI improvement occurred in both interventions. Blood pressure and heart rate were positively associated with the low-fat diet group, yet lipid and glucose regulation markers improved similarly in both. These findings are contrary to the background patterns seen in non-intervention patients, whereby cardiovascular health, glucose regulation and lipid metabolism become increasingly dysregulated over time. A nutritional education programme modelled on the interventions in this study that incorporated family support may therefore improve transplant outpatient care.

5.2. Comparison of Findings with other Reports and Studies

What is eaten on a daily basis contributes greatly to the development and progression of post-transplant related disorders. From a dietary perspective, patients are all too often told what to do, but far too little emphasis is placed on the importance of initiating those changes. This study sought to address this imbalance by providing the prerequisite tools and support for initiating new dietary habits. Distinct peri-operative nutritional advice has for many years been based on British Heart Foundation (BHF) low-fat guidelines. Following this, advice is only provided on an ad-hoc outpatient basis. At the time of writing, a specific literature search for relevant post-heart and lung transplant Mediterranean and low-fat diet studies yields a total of 3 research papers; none of which are recent. Considering the current wave of diet-related health issues such as obesity, CVD and diabetes occur with greater frequency in
these distinct patient populations, re-empowering patient decision-making processes may offer at least two distinct advantages. Firstly, encouraging and supporting healthy lifestyle habits provides patients with the practical skills to directly influence their own health. Secondly, adoption of a healthy dietary pattern is highly likely to mitigate long-term healthcare expenditure costs [294, 295].

A primary question we sought to address was ‘feasibility’, without which the prospect of future implementation would be unattainable. Our findings reveal that implementation of a dietary education programme was feasible at the UHSM transplant outpatient centre. The practicalities of running group training sessions and outpatient consultations were achieved with considered planning and close involvement with clinicians and staff. By closely liaising with participants and family members, many logistical fears did not materialise. For instance, conducting the training sessions on consecutive days ensured attendance flexibility and minimised participant absence. Block training sessions also maximised the use of human and material resources, which are an important implementation consideration. Telephone contact required a flexible approach to ensure participants were adequately supported. In general, the allotted discussion time averaged 10-15 minutes per call, though occasionally, additional time was provided for further support. In addition, individualised text messaging proved a simple and effective measure for reinforcing clinic appointments, fasting requirements and questionnaire returns.

It is true that prior to commencement many patients expressed distinct interest for MeDiet enrolment when canvassed; however, overall retention rates in both intervention groups who were otherwise treated identically remained high. Whether the low attrition is specific to heart and lung transplant patients is not easily ascertained as dietary studies in these two distinct populations are lacking. In comparison, when similar analysis was conducted in the large PREDIMED RCT, low-fat and MeDiet attrition at year 2 was 11.3% and 4.9%, respectively [9]. This effect was more pronounced in younger subjects with higher BMIs, which may reveal why one MeDiet obesity study reported 32% dropout rates at 12 months [296]. Whilst our findings were ascertained at 12 months, attrition was much lower and did not differ between interventions. Moreover, baseline BMI in this study was approximately 1kg/m² less than the PREDIMED participants, placing our groups in a comparable ‘upper overweight’ bracket.

The evolutionary advantage of consuming calorie-rich foods during periods of less abundance and increased physical activity is well understood. Unfortunately, when these
foods are freely available, it requires greater psychological discipline to choose healthier options and limit portion sizes. This survival mechanism exemplifies why dietary compliance rates are highly variable and brings us to the crucial question of ‘adherence’. Anthropometric changes such as weight, waist and BMI enable physiological adherence to be evaluated, but emphasis was also placed on comprehensive and group-specific questionnaires. Isolated FAQ and combined FFQ scores suggest the total change in adherence was significant in both interventions. However, overall adherence was more robust in participants following the MeDiet. A combination of two differing questionnaires allowed dietary change to be compared and contrasted, whilst providing a tool to focus awareness on eating habits. A food diary may have focussed awareness further, but in this study would have placed additional pressure on patients already burdened with comprehensive medication regimens. The validation results performed early in the study with non-intervention heart and lung transplant recipients showed the effectiveness of these measures in these patient populations. Furthermore, the 9 and 14-point FAQs allowed immediate interpretation of answers during follow-up consultation. Supportive advice was then provided for missing or under-consumed food groups.

Under or over-reporting remains a potential concern when participants rely on memory recall. Immunosuppressive regimens may contribute to impaired post-transplant cognitive function [297, 298] and have been considered. The questionnaire results may have been expected to coincide with greater weight and BMI reduction in both interventions, but they occurred modestly in the MeDiet group only. Reported adherence was slightly stronger in the MeDiet group and may partly account for this effect, but recognition that baseline adherence scores were higher for low-fat participants may actually stem from peri-operative dietician advice. Nonetheless, it must be remembered that the two FAQs were not directly comparable.

Both interventions demonstrated a reduction in waist size at week 25, yet weight and BMI remained unchanged in the low-fat group. Despite this outcome TG levels decreased significantly in both groups throughout the study; an effect that would not be expected to coincide with general caloric increases. Glucose regulation also improved in both groups thus further consolidating our compliance outcomes. The benefits of weight reduction in overweight and obese individuals are less defined in transplant populations, but have been extensively demonstrated in other patient groups [299]. However, the post-transplant convalescence period is highly variable and may differ significantly between heart and lung transplant recipients. The ability to return to daily activity influences caloric output, and
subsequent inactivity compounds the loss of lean muscle mass at the expense of fat mass. Physiology is further altered when metabolic demands change with age, thus contributing to sarcopenia, which to greater or lesser degree is controlled by resistance exercise [300]. Transplant recipients often face a chronic decline in health and mobility (deconditioning) during the pre-operative period. Inactivity is further exacerbated during the long peri-operative period, whereby the mean length of hospital stay is 24.4 days for heart [301] and 18 days for lung recipients [302]. A further 3-6 months are generally required to return to health, though this varies greatly depending on the individual. These aforementioned points are important as they have the ability to influence the results of this study.

In spite of the lack of significant weight loss, neither intervention resulted in any weight gain, and the MeDiet group decreased moderately over 12 months. We must consider that various post-transplant factors work in tandem to negatively promote long-term weight gain, and these occur in spite of peri and early post-operative dietician involvement with general low-fat diet advice. Therefore our findings would not be expected to follow negative prevailing outpatient trends, thus the likelihood of participants in both groups losing weight without intervention involvement was improbable.

The MeDiet group were much less constrained in regards to mono- and polyunsaturated fat intake. Our weight outcome in this group is in keeping with the large PREDIMED study, which did not report weight loss in either the Med or low-fat intervention groups in its 5-year results, though data provided at 1 year highlighted a -0.3kg and -0.7kg reduction in MeDiet (EVOO) and low-fat groups respectively. Compared to our participants, their low-fat control group displayed greater weight loss, but our MeDiet group completed the study with a 1.5kg greater reduction than in PREDIMED [303]. Neither this study, nor PREDIMED were constructed as weight loss programmes per se: both sought to evaluate a range of health parameters. Unlike the PREDIMED study our low-fat group achieved significant changes in dietary adherence scores, which then prompts the question, why was weight loss not achieved?

One explanation is a lack of accurate adherence questionnaire reporting. The need for honest reporting was clearly stipulated to each participant at all time points. However, questionnaire response bias was determined prior to participant recruitment and was within agreeable limits. Therefore, the data does not suggest that food intake responses were misleading in either MeDiet or low-fat diet groups. Another possibility is that in some individuals a return
to health may have led to an increase in lean peripheral muscle mass, which would offset losses in body fat. This effect may have been more marked in wave-2 as it contained more newly transplanted individuals. To counterbalance this argument, high glucocorticoid intake has been shown to inhibit muscle protein synthesis and subsequently induce myopathy [304]. However, in this study prednisolone dosages at 52-weeks were tapered across the MeDiet and low-fat interventions by -18% and -19%, respectively. Even withstanding this reduction, chronic high-dose steroid medication alters fluid and electrolyte balance and increases gluconeogenesis, thus contributing to weight gain and adipose redistribution [305]. Interestingly, it was not uncommon for a number of participants to discuss the effects of abdominal fluid retention during consultation. Waist circumference decreased significantly at 25 weeks, which in lieu of generally increasing post-transplant trends is a positive observation. However, given that the incidence of high CVD and type-2 diabetes risk correlates with a waist circumference of ≥102cm in men and ≥88cm women [306], our 52 week findings justify a greater drive for reduction.

Mean BMI in both groups remained outside of healthy range throughout the study. Nonetheless, no further increases occurred during the intervention; unlike the increasing pattern encountered in other observation studies [84, 227]. This last point is further strengthened with our preliminary assessment findings at week 25. Both intervention groups significantly improved BMI and weight parameters when compared to evenly matched non-intervention patients that were eligible for this study. Moreover, no general stipulations were made for calorie counting or physical activity. It may therefore be a misconception to attribute the MeDiet with weight reduction. Higher-fat diets are known to contribute to satiety, but the high calorie content of EVOO has not been associated with weight gain in MeDiet studies supplemented with olive oil and nuts, rather the effect infers weight buoyancy. This result might be satisfactory for patient populations occupying healthy BMIs, but for individuals outside this range, clearly further modifications are required, such as caloric reduction and individualised tailoring of advice.

The investigator delivered advice regarding adoption of both interventions in as holistic manner as our teaching environment would allow. This was easily achieved within the UHSM clinical setting and required no special amenities or equipment. It is feasible that other transplant clinics could adopt a similar intervention with considered planning.

By encouraging participants’ family members to take part, it helped foster deeper patient support at home. During discussions, it became clear that food preparation was conducted by
patients and partners’ alike and so overall adherence would have been significantly affected without the assistance of many patient partners. In some cases, a professional relationship was also built with these household members and they themselves became primary mediators for receiving and conveying telephone and consultation advice. The incorporation of the household member questionnaire sought to gather feedback from this area of the study, and it revealed the benefits of taking part in the teaching session were not solely patient-based. In addition, printed information designed to accompany each intervention offered new ideas and recipes, whilst reinforcing advice provided during the tuition session. The low-fat group received an additional recipe book, whereas the MeDiet group were provided with a 3 month supply of EVOO. Whilst the book’s contribution to low-fat adherence could not be quantified, the EVOO undoubtedly improved olive oil intake scores.

Progress is rarely achieved in isolation and the assistance and support of a trained chef was testimony to this point. The successful planning and execution of the training demonstrations were major parts of the chef’s practical contribution, which was also reflected in the anonymous feedback forms. His personal journey to health through dietary and lifestyle change was woven into the cooking session. This further humanised each talk and highlighted what can be achieved with reprogramming of old habits. All the foods on show were plant-based and fresh or minimally processed. Animal foods were discussed, but kept to a minimum as plant-based foods are the foundation of a healthy diet [307] and often most lacking. By incorporating various cooking and preparation techniques, participants were provided with strategies for putting the information into action. The chef’s ability to clearly articulate the core message played a key role in overall outcomes. During the demonstration session the investigator also strived to convey the importance of various food groups in relation to disease progression. Reducing long-term complications requires an individual to take control of their own health, and is especially true of CVD. The cardiovascular findings produced by Moriguchi et al epitomise the adage ‘genetics load the gun, yet lifestyle pulls the trigger’ and this point was highlighted during the baseline talks [308].

Prediction of future cardiovascular events depends on anthropometric and bio-clinical measurements as well as blood pressure, though blood pressure and heart rate share an irrefutable connection to CVD. From a cardiovascular risk perspective, both interventions were characteristically well matched. Therefore, our prospective findings suggest improvements were more evident in the low-fat arm and occurred independently of antihypertensive medication. Notwithstanding statistical significance, the cumulative
Cardiovascular risks following transplant are great and the clinical implications deserve further consideration. As previously discussed, small changes in heart rate, systolic and diastolic BP may translate into considerable patient benefit. A systolic rise of 1mmHg was associated in one Mediterranean diet study with an increased major cardiovascular event risk of 1-2% [216]. Our findings were in many cases similar to, or even exceeded the outcomes of PREDIMED, DASH or Lyon Diet Heart studies. Of the 20 heart transplant participants analysed at the end of intervention, 2 remained free of antihypertensive medication throughout, compared to 5 out of 19 lung transplant participants. As would be expected, heart recipient antihypertensive dosages were higher overall. In contrast, wave 1 and 2 low-fat lung groups contained individuals prescribed the lowest doses. Drug type and dose remained largely unchanged in both interventions. In contrast, MeDiet BP and heart rate remained relatively unchanged throughout the study. In the absence of changes, it should be noted that measurements were within clinical range and no further deterioration occurred.

Blood pressure denotes primary cardiovascular fluctuations, but when combined with biochemical markers, the diagnostic value increases greatly. Considering homeostasis is continually disrupted post-transplant, subtle changes in multiple clinical biomarkers may translate into an effect of greater magnitude. Our lipid findings exemplify this point. The Lyon MeDiet study reported that the risk of a further coronary event increased 20-30% with each 1mmol/L rise in Tchol [216]. Lipid metabolism becomes increasingly dysregulated following transplant, yet our findings indicate Tchol decreased similarly by 0.56 and 0.4mmol/L in the MeDiet and low-fat groups respectively. This observation is interesting given the disparate dietary fat advice offered to each group. In actuality, the cardiovascular benefits attributed to a traditional MeDiet have been largely independent of cholesterol reduction and may owe more to the synergistic composition of plant-based nutrients [309]. The Lyon Heart and PREDIMED studies both attest cardiovascular risk-reduction outcomes, but do not coincide with a significant fall in cholesterol. In this current study, Tchol significantly decreased in both groups over 52 weeks to produce similar measurements. Considering the core advice offered to both interventions was essentially the same, it further substantiates the view that the foundation of a healthy diet is plant-based [307].

Significant improvements in serum TG did emerge as a clear beneficial clinical outcome in line with the positive adherence scores. Even factoring in questionnaire reporting error, this suggests participants in both interventions reduced their intake of surplus calories and this was achieved without calorie counting. Placing this into perspective, the PREDIMED study
reported significant 1 year TG decreases in the MeDiet nut intervention group only; -0.17mmol/L versus -0.09mmol/L low-fat control [303]. In comparison, our MeDiet group (EVOO and nuts) declined by -0.17 and the low-fat group by -0.44mmol/L. TG values share a close association with HDL; a decrease in the former tending to produce a rise in the latter [310]. However, HDL remained relatively static in both interventions. Considering the desired clinical aim is ≥1.2mmol/L, both interventions retained values that are considered more cardio-protective. The very low-fat Ornish diet is perhaps one of a few primarily plant-based diets proven to substantially reduce Chol and LDL fractions, whilst not affecting HDL [311]. The Ornish diet may offer significant cholesterol reduction benefits, but the MeDiet seems to be more effective when it comes to adherence. Whilst our study did not necessarily stipulate a ‘very’ low-fat intake, it does appear to attest greater adherence in participants assigned to the MeDiet group.

Most LDL lowering strategies tend to centre on statin medication for CVD risk reduction. Unfortunately, even with adequate treatment, significant residual risk often remains and this may in part be attributable to LDL particle density [312]. It should be noted that in this study, LDL was estimated and not directly measured. Furthermore, whilst lipoprotein particle profiling was not performed, it appears that measurement of LDL particle size and number, and serum apoplipoprotein-B may yield stronger prognostic value for future cardiovascular events [313]. Nonetheless, MeDiet baseline LDL was 3.4mmol/L and therefore constitutes increased clinical risk, yet at 52 weeks measurements were lower than this threshold (2.94mmol/L). In contrast, the low-fat group remained under this value throughout the study. Reported red meat intake declined in both interventions throughout the study, and the decrease in LDL and circulating IGF-1 may substantiate this effect. Our advice encouraged both groups of participants to substantially increase vegetable and fruit intake at the expense of refined carbohydrate. The questionnaire data suggest fibre intake increased significantly in both interventions, and higher intakes are associated with improved blood lipids and CVD reduction risk [309, 314]. Additionally, increased fruit and vegetable intake is correlated with improved gut microbiota health and a reduction in CVD risk factors [315].

CVD risk profiles were also explored from another perspective by calculating lipid ratios. The most notable observation occurred with LDL/HDL which showed a positive inverse association in the MeDiet group only. Further improvements in Tchol/HDL ratio also favoured the MeDiet group. However, the TG/HDL ratio improved similarly in both interventions. This suggests that overall, improvements in lipid profile favoured the MeDiet
group, but again this needs to be put in context of overall primary clinical signs and measurements. Each intervention demonstrated a reduction in cardiovascular risk, but from differing perspectives. Acknowledging that baseline BP and heart rate were clinically well managed in both dietary groups suggests one strategy could not be favoured in regard to improved cardiovascular benefit over the other. In light of the overall improvements in Tchol, LDL, TG and minimal change in HDL, which incidentally was within range in both interventions, the low-fat group may actually have demonstrated a slight advantage in this prospective study. Unfortunately, the hsCRP results did not reveal any further predictive information that would favour one intervention over the other. Elevated hsCRP is associated with increased CAV development [316] and adverse lung transplant outcomes [317], but in a bracket beyond our measurements. Nonetheless, the mean ranges occupied for this inflammatory marker remained within a moderate risk category (1-3mg/L) throughout our study [318]. It must also be acknowledged that both dietary groups contained a number of patients experiencing inflammatory events at final follow-up; therefore, its predictive value is difficult to define in a small study sample.

The glucose regulation data revealed improvements occurred similarly in both groups. Fasting glucose decreased by -4.8% in the MeDiet and -5% in the low-fat group, with a respective -11% and -8.7% decrease in fasting insulin. Whilst these changes correlate with improved dietary compliance, it must also be acknowledged that tailored prednisolone reduction may also have influenced the glucose outcomes. On the other hand calcineurin inhibitor and mycophenalate mofetil doses remained consistent, and both classes of drug contribute to insulin impairment [319]. When insulin resistance was calculated with HOMA2 modelling, it coincided with respective -12% and -9.8% decreases in MeDiet and low-fat values. In contrast, insulin sensitivity increased by -13.2% and -10.3%, respectively. This indicates insulin uptake and utilisation were more effectively regulated at the end of trial. However, comparison with a large non-transplant population study suggests insulin resistance and sensitivity values continued to occupy ranges associated with increased T2D risk [320]. Improved glycaemic control was attained similarly with both diets, but our findings highlight β-cell output was greater overall in the low-fat group. Relevant transplant-related studies are lacking, but given the literature defines steady-state β-cell function at 100% in normal young adults, our findings appear to suggest a degree of impairment. This also fits the HbA1c data which corresponded with the NHS ‘upper normal’ guideline range. Since diabetes incidence following transplant greatly affects patient life expectancy and medical costs alike [321], curtailing the diabetogenic effects of immunosuppressive medication undoubtedly lies in
careful drug monitoring and tapering, combined with a lower glycaemic eating pattern as advocated in both interventions [322]. Age-related disorders such as diabetes, cancer and cognitive decline share a close association with accelerated telomere shortening and cellular senescence [323]. The role of nutrition and its contribution to healthy aging have been observed with adherence to a Mediterranean eating pattern [324]. In this study participants reported an increase in total vegetable consumption, whilst reducing meat and dairy intake; other studies demonstrate this pattern is associated with increased leukocyte telomere length [325]. Nevertheless, accelerated telomere reduction correlates with allograft aging [326], and all-cause mortality in non-transplant subjects aged over 60yrs [327]. IGF-1 displays a wide range of pleotropic actions, however excessive secretion has been linked to telomere shortening [328], whereas cancer and diabetes mellitus protection are reported in populations with reduced IGF-1 [329]. IGF-1 decreased significantly in both our dietary interventions, and whilst other relevant transplant-based studies are lacking, the values exhibited here remain within healthy range when we compare to age-matched background values. The reduction of IGF-1 in both our groups was similar, and would correlate with increased vegetable intake and lower red meat and dairy consumption. Considering transplant recipients are at increased risk of accelerated aging, cancer and diabetes, reductions in circulating IGF-1 in this study may offer clinical benefit.

It is duly noted that our results require cautious interpretation as prednisolone dose decreased in both interventions during the study and may have contributed to improvements in glucose regulation, and changes in IGF-1 values. However, diabetes development increases significantly in heart and lung recipients within the first two years following transplant (discussed in section 1.5.2), an effect that was not observed during this 12-month intervention.

Minimising future transplant-related complications is of great importance. Whilst lifestyle choices are patient-mediated, if we as practitioners fail to optimise care during the post-transplant period, it undermines the extensive team effort that culminates in a successful transplant. Positive lifestyle advice is perhaps more prevalent now than in any other time in history, but the rise in chronic lifestyle-related health conditions clearly demonstrates that this knowledge fails to be implemented on a consistent basis. It is proposed herein that it may be more efficacious to implement a comprehensive lifestyle training strategy early, or even prior to transplant to encourage patients to set off on the right foot. The axiom “an ounce of prevention is worth a pound of cure” is especially true of lifestyle choices. However, it would be erroneous to suggest changing the habits of a lifetime is straightforward [330].
Nevertheless, the results of this study, and others discussed throughout this thesis, identify that future health risks can be ameliorated with a concerted, patient-centred approach. Considering the small sample size of this study, the findings offer distinct clinical relevance that may also offer translatable benefit in other chronic disease states. Conducting the programme from a holistic perspective offered many advantages, not least, the importance of why patients should improve and maintain healthy eating habits, whilst offering practical tools for implementation [331]. More importantly, by maintaining frequent contact it helped establish a discursive relationship whereby positive incremental dietary changes were reinforced. Short-term evaluation of post-study adherence indicates participants in both interventions continued to follow healthier dietary habits.

If one asks the question ‘could the current BHF low-fat strategy be improved?’ the research literature and findings from this study suggest the answer is ‘yes’. If we acknowledge each diet offered generalised benefits, then it may be efficacious to combine elements of each into a more integrated and tailored future strategy. As previously stated, transplant medication is ironically a significant contributing factor to long-term health complications and remains outside of patient control. In contrast, patient empowerment encourages individuals to take responsibility of behavioural patterns, with the aim of mitigating disease progression [332]. This study therefore provides evidence that implementation of a holistic training programme is feasible in a heart and lung transplant outpatient setting.

5.3. Strengths and Weaknesses of Study

This prospective feasibility trial demonstrates various strengths and weaknesses. A combination of detailed FFQ and group-specific FAQs ensured compliance data were robust, by enabling identification and comparison of adherence to specific food groups. In addition, the inclusion of an FAQ allowed the investigator to quickly determine dietary adherence during outpatient consultation, and where necessary, make adjustments. Unfortunately, the inability to quantify the excretion of polyphenol adherence markers restricted the initially proposed objective compliance assessment.

Wave-1 serum samples were screened for a comprehensive panel of circulating steroids. Unfortunately the results indicated most analytes remained below the limits of quantitation, thus rendering the results inconclusive. Suppression of the hypothalamic-pituitary-adrenal (HPA) axis in this study was most likely attributable to chronic prednisolone treatment [333]. Constructing the study from a holistic perspective ensured participants gained a more
comprehensive understanding of the physiological importance of food, in combination with practical skills. This study differed from a conventional approach in its flexibility and support skills, as a narrow approach often limits communication. The study sought to broadly educate about long-term illness and the positive role of diet through patient empowerment. Moreover, the investigator encouraged household member involvement to bolster patient support. Our approach may ultimately require practitioners to spend more time and resources than a conventional model. This could be viewed as a limitation because it incurs financial outlay in the short-term. However, the long-term advantages of promoting healthy eating positively decrease downstream health care costs and re-empower patients as was shown here. Material expenditure and time management were more effectively optimised when tuition sessions were performed in concentrated block sessions. However, this approach would not work efficiently if performed on an ad hoc basis. Conducting teaching seminars in groups served to consolidate a supportive group environment, which may offer complementary benefit in the context of a post-training support group.

Although target recruitment figures of 40 heart and 40 lung transplant recipients were not achieved, attrition rates were exceptionally low, which in itself was an indicator of intervention feasibility and acceptability. Mediterranean and low-fat eating patterns served as effective comparators as the latter forms the basis of UHSM transplant dietetic advice. By modifying this advice the investigator encountered two key issues. Firstly, the advice offered to the low-fat group was distinctly different to the BHF advice but foundationally similar to the MeDiet intervention; the basis for healthy eating is rooted in an unprocessed, plant-rich diet. Therefore, potential changes between group outcomes may have been somewhat limited. Secondly, to combat this, the study may have theoretically benefited from a third group containing consented individuals who were prepared to partake in the study and provide the same biological, anthropometric and subjective data as the intervention groups, without making any additional dietary changes (similar to the week-25 weight and BMI assessment). This would have provided a non-intervention comparator to gauge general outpatient trends. The PREDIMED researchers assessed the low-fat group’s MeDiet adherence score. In this blinded RCT, it was felt that withholding the information would reduce bias as many patients expressed interest for being assigned to the MeDiet group. Nonetheless, assessing the low-fat groups MeDiet score at baseline and 52-weeks may have been advantageous. Fat intake was not explicitly measured, without which the fat content and volume of individual foods could not be accurately predicted. However, this remained outside the remit of this study.
A key strength of this study was encouraging household members to take part and support participants. Not all individuals were accompanied by a partner, but those who did appear largely supportive, and in some instances made significant changes to their own dietary habits. Both groups contained a larger proportion of males (representative of the UHSM transplant population), many of which had female partners who reported that after visiting the training seminar were motivated to prepare healthier meal options. Moreover, outpatient and telephone contact helped build a rapport that consolidated the study information. One potential limitation of this personal approach is that the allocated 10 minute duration for telephone contact sometimes extended further.

Activity levels are inextricably linked with composite health outcomes; however, physical activity was not evaluated in this prospective study. An extensive range of validated physical activity questionnaires are available, and had they been incorporated into this study, would have provided useful subjective data. Furthermore, observation of activity levels would have potentially enabled transplant-specific changes to be attributed to an increase, or lack of exercise. However, this was outside the logistical capability and scope of this prospective study.

A final, but perhaps most crucial consideration was that the training and motivation of the lead investigator may be difficult to replicate on a large scale e.g. in a phase III trial. A holistic nutritional understanding was necessary. While this study was not necessarily unique, it differed in the way the interventions were conveyed with tuition by a chef and intensive follow-up, and these may be difficult to scale-up with finite resources.

5.4. Adverse Events

No adverse events or reactions were reported during the course of this study. One participant died after 7-months of study participation owing to chronic lung rejection. EGFR rates remained above limits; therefore no participants were required to undertake a hypokalaemic diet, which on the grounds of potassium reduction would have necessitated study exclusion.

5.5. Implementing the Study Framework

As raised above, the findings produced by this prospective study demonstrate the feasibility of small-scale implementation in a heart and lung transplant outpatient setting. If we were to consider initiation of a second phase, the most pertinent questions would be as follows:
1. How practical would it be to conduct a large-scale UHSM or nationwide study?
2. Cost-effectiveness of such a programme

Since most of the infrastructure and clinical support is already in place at UHSM, rolling out a similar outpatient programme would be practical at our centre. Logistical considerations are negligible as patients would be coached on an outpatient basis, with additional support conducted via telephone. Additional visits to the centre would be solely for the initial education seminar and a proposed periodic refresher session. The implementation of a multi-centre study however would require considered planning to ensure close partnerships were forged. Failure to engage effectively with other care providers would dilute the core educational message.

In answer to the second question, the programme must be economically viable if it is to be considered on a larger scale. Within a transplant setting, secondary complications are experienced by most, if not all patients. Therefore, strategies that positively influence disease progression offer many advantages, not least, long-term cost reduction. Transplant clinicians increasingly face significant challenges in their patients such as managing impaired BMI, glucose and blood lipid regulation, which may be mediated by modification of habitual dietary patterns. Promoting cost-effective healthy lifestyle changes offers the added benefit of re-empowering patient decision making processes. Conducting training sessions in a group setting would reduce ancillary costs required for educational food items and chef fees. Additional research costs would incorporate bench fees for necessary biochemical investigation and disposable items.

5.6. Conclusions

In conclusion, there remains a nutritional education shortfall during the transplant outpatient period. Reasons for this are varied, but understandably, resources are primarily focused on pre and peri-operative patients. Nutritional choices are key factors in causing obesity and non-obesity related conditions. Metabolic disruption by immunosuppressive medication further increases the likelihood of developing complications following transplant. Not only does this directly affect patients, it financially burdens transplant outpatient departments and the NHS at large. The results from this study demonstrate two distinct positive outcomes. Firstly, implementation of an energy-unrestricted Mediterranean and low-fat training programme is feasible in a heart and lung transplant out-patient setting. Secondly, adherence
and clinical outcomes between MeDiet and low-fat groups remained marginal; in actuality the two interventions were foundationally the same, and may have partly explained the similarity in composite endpoints between groups. Adoption of new dietary habits is achievable when patients and family/household members are constructively guided and supported. Empowering patient decision-making processes in a real-world setting has far reaching health consequences that offer the potential to improve long-term disease development and progression.

5.7. Contributing Authors

Authors contributing to this body of work are as follows; JF, TE and AG were closely involved with study design and structure. TE was responsible for data collection and laboratory analysis under the auspices of the UHSM biochemistry department. KM performed statistical analysis of adherence data, and JM conducted statistical analysis of biochemical and anthropometric measurements. The first thesis draft was written by TE, and subsequently critically reviewed and revised by JF and AG.
References:


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139. NICE Guidelines. Cardiovascular disease: risk assessment and reduction, including lipid modification. 2014 Clinical guideline [CG181].


Appendix A. Patient information sheet

Title “Assessment of The Mediterranean Diet in Heart and Lung Transplantation – AMEND-IT”

You are being invited to take part in a research study organised by The Transplant Centre. Before you make a decision regarding your participation in this study we would like you to understand its purpose and details. Please read the following information carefully and if you should feel you require further information or clarification, please do not hesitate to ask us.

What is the purpose of this study?
The purpose of this study is to identify if either a low-fat or a modified diet based on foods readily eaten in the Mediterranean region, affects immune function and the process of inflammation. Inflammation is a key mechanism through which organs reject. Depending on the type of foods we regularly eat, our diet can either provide essential nutrients required for daily function, growth and repair (for example fresh, unprocessed food sources), or it may incorporate food types that are known to contribute to the development of many inflammation-driven diseases (for example refined and/or processed foods). Many ingredients included in a Mediterranean-inspired diet have demonstrated anti-inflammatory properties in a number of research studies, and may positively influence immune function. Similarly, a low-fat diet offers many health benefits and is viewed as best general advice. Our aim is to identify if both diets, which are rich in beneficial nutrients, can improve health following heart and lung transplantation.

Why am I being asked to take part in this study?
You are a patient who has received a heart or lung transplant and are cared for by The Transplant Centre at Wythenshawe Hospital.

Do I have to take part?
It is up to you to decide whether to participate or not. If you do decide to take part you will be given a consent form to sign, but you are free to withdraw your consent at any time. The Wythenshawe team will give the best possible care regardless of whether you decide to take part or not.

What is involved? What will happen to me if I take part?
If you decide to participate in the study you will first be asked to sign a consent form. Following this, you would be randomly assigned to one of two groups. Both groups will be provided with regular tuition and support to help gain an understanding of the health benefits of either diet. It is necessary to determine if either diet can reduce the effects of inflammation and improve a range of other health parameters, as a low-fat style diet is currently recommended as the best advice following transplant surgery. The decision determining which group you will be assigned is not made by the researchers, it is performed electronically to minimise bias when analysing the results at the end of the study. Determining what effect diet has on your health will require both diet and control groups to provide two additional fasting blood samples (10 millilitres or two teaspoons) and one small urine sample (10 millilitres). The blood samples you provide will be taken during routine sample collection as an in/out patient and will not require additional needles to be inserted. Samples would be taken at 4 time points (week 0, 6, 25 and 52) and must be provided following an overnight fast, as many of the analysis techniques require it. In addition, your weight, waist measurement and height will be recorded. Tick-box style diet questionnaires will be conducted at specific time-points; these help capture your diet information and any subsequent changes over the 12 month period. No additional interventions will be required.
**How long is the study period?**
The study duration for both the modified diet and control group is 12 months in total. At the end of this study, continued dietary support will be available should you require it. No further blood samples, measurements or questionnaires would be required at this point.

**What will happen to the samples I give?**
The blood samples will be used to analyse the cells of your immune system, measure cholesterol, insulin, glucose and level of hormones linked to inflammatory processes. Urine samples will be used to measure intake of dietary fruit and vegetable polyphenols. Samples will be coded and not contain any direct information that associates you with them. With your permission they can be stored for future research at the Trust.

**What are the risks of the study?**
The blood and urine samples that you provide for this study involve minimal risk to you since they would be taken during your routine procedures. You will not be exposed to any additional or unnecessary risk for the purposes of this study.

**What are the potential benefits of the study?**
The types of foods you eat have the potential to not only alter the incidence of inflammation, but also alter the development of a range of health complications commonly encountered following transplantation. Strategies to reduce inflammation in heart and lung transplantation are of paramount importance. The information generated during this study may help us understand the influence of diet in transplantation immunology and improve the development of future transplant treatments.

**What are the indemnity arrangements of the study?**
The University Hospital of South Manchester NHS Foundation Trust has agreed to take the responsibility to supervise that progress of the study is scientifically and ethically adequate. According to the NHS indemnity scheme, the South Manchester University Hospital NHS Foundation Trust is responsible for safeguarding you. If necessary, the NHS would cover the costs and damages of any unintended failure to protect your health care interests. If you have any complaints about the conduct of the study, you may contact the National PALS (Patient Advice and Liaison Service) Network at Wythenshawe Hospital at: East Entrance, New Acute Block, Southmoor Road, Wythenshawe, Greater Manchester M23 9LT (0161 291 6611).

**What will happen if I refuse to take part in this study?**
It is your choice to participate in this study and we will completely respect your decision. There will be absolutely no change in the way you are treated and you will receive the best possible care by the Wythenshawe Hospital team.

**Can I change my mind about taking part?**
Yes of course. If you decide you no longer want to participate in this study, we will stop taking additional blood and urine samples immediately. However, we will ask for your permission to use the data generated (excluding any personal details) from the samples that would have been collected. Just inform the doctor or nurse during your next visit.

**How can I obtain more information about this study?**
If you would like to receive more information on this study, please call Timothy Entwistle, AMEND-IT Research Lead (0161 291 2281) or James Fildes, Principal Research Scientist (0161 291 5023) or you can contact the Research & Development Department at Wythenshawe Hospital.

**What will happen with the results of the study?**
The results will be published in the Transplant Centre’s periodic newsletter “InTouch”, as well as scientific journals. However, all participants’ details will remain anonymous. Please feel free to ask about any of the results during your follow up visits.

Thank you for considering your participation in this study.
Appendix B. Study recruitment letter

Mediterranean Diet Study

AssessMent of the mEditerraNean Diet In Heart and Lung Transplantation
(AMEND-IT)

Dear ……

My name is Tim Entwistle and I work as part of the transplant research team. My area of interest is nutrition and the effect food has on inflammatory processes involved in disease. Inflammation lies at the heart of many illnesses; it plays a crucial role in the development of disorders ranging from hay fever to heart disease, arthritis to cancer. More importantly, inflammation is directly involved in graft rejection.

Diet & Disease: The dietary choices we make are important to our health, as all foods are not created equal. Depending on the food type and preparation method, they either contribute to improving or maintaining your health, or they drain the body of vitality by making it work harder to build healthy cells and perform its daily housekeeping chores. A healthy diet is therefore of huge importance following transplantation as immunosuppression can leave patients at greater risk of developing a range of health disorders.

There is significant evidence demonstrating you are what you eat, whereby the risk of disease can be altered by diet. Research demonstrates that rather than undertaking the latest ‘fad diet’ or eating a new ‘super-food’, we need to eat a varied range of unprocessed fresh foods, and just as importantly, aim for consistency. Interestingly, there are many foods that do offer health benefits and by incorporating them into a balanced diet, it allows them to work together, whereby the combined effect is more beneficial.

Mediterranean Diet: One type of eating pattern that is thought to be beneficial is a Mediterranean-style diet. Eating this type of diet involves preparing fresh unprocessed meals, rather than processed foods, and increasing the quantity of fresh fruit and vegetables on your plate at each meal. Choosing whole grains over white processed products ensures essential vitamins, minerals and fibre are retained; whilst regularly eating portions of fish and seafood that contain anti-inflammatory fats, helps reduce the incidence of heart disease. Red meat is eaten occasionally, and saturated fats, margarines and sunflower oils are replaced by extra virgin olive oil which is used for cooking and dressing foods. This type of diet has been shown to alter the development of inflammation-related diseases; many of which can affect your health following transplantation.

AMEND-IT Study: We are planning a new research programme (AMEND-IT) looking at the effect diet has on inflammation, but we need your help. The study has been funded by the New-Start Charity and represents a scientific first in this subject area. Our aim, with your help, is to gain an understanding of how the foods you eat on a daily basis relate to your health and wellbeing. Research of this type requires 2 randomly-allocated groups to be studied; one randomised group will follow a modified diet, the other (control) will eat a low-fat diet as advised by our transplant dietician. By studying two such groups, it will help our researchers compare and contrast any differences between people eating a modified diet and a low-fat eating pattern. If you are interested in taking part you will be randomly allocated to one of these two groups. This ‘randomisation’ is essential to remove bias and make the study outcome more representational. Our research team will study and compare a variety of factors in blood and urine which are related to
foods eaten by both groups, and how they relate to chronic inflammation and general health.

If you are interested in taking part, please complete the attached form below and return to the Transplant centre. If you would like more information, please email or phone the number below and we will get back in contact. Returning the form commits you to nothing, but will help us assess the level of interest. It is proposed that when all the forms have been returned, we will set a date to hold a meeting/presentation at the centre for everyone who has indicated an interest. You can meet the team and gain a better understanding of what is involved. Conducting this type of research is essential for understanding how diet affects your health following transplant.

Please could you cut & return the form below to the Transplant Centre in the self-addressed envelope provided by __/__/14. This will allow us to properly assess who is interested (or not) in taking part in the AMEND-IT diet study.

For further details, please contact: Tim Entwistle

Tel: 0161
Email: timothy.entwistle@postgrad.manchester.ac.uk

Would you be interested in taking part in our Mediterranean diet study?

No ☐
Yes ☐

Name: _____________________________________________

Contact number:
Home ________________________________
Mobile: ________________________________
Email: ________________________________
Appendix C. Inclusion and exclusion screening questionnaire

Date: _ _ / _ _ / 20 _ _
Surname: ___________________________________
First name: ___________________________________
Date of birth: _ _ / _ _ / _ _ __
Address: _____________________________________
________________________________________________
Postcode: _____________

Telephone: ___________________________ Mobile: 0
Landline: _______________ 

Transplant group:
Heart ☐ Single lung ☐ Double lung ☐

Sex: Male ☐ Female ☐

Are you following any pre-existing medically prescribed diet?
☐ Yes ☐ No

Are you registered diabetic?
☐ Yes ☐ No

Do you have any known kidney disease?
☐ Yes ☐ No

Do you have any known food allergies?
☐ Yes ☐ No

Can you cut your intake of animal/saturated fats; butter, lard, pastries, hydrogenated fats/spreads? Are you willing to try?
☐ Yes ☐ No

Do you eat a fibre-rich diet i.e. abundant in fruit, vegetables, whole grains and legumes? If not, are you willing to try?
☐ Yes ☐ No

Do you eat fish/seafood (especially oily fish)? If not, are you willing to try?
☐ Yes ☐ No

Would you cut your intake of red meats? Are you willing to try?
☐ Yes ☐ No

Weight: _______ Kg
Height: _______ Cm
BMI: _______
Appendix D. Food frequency questionnaire

Mediterranean Diet Study
AMEND-IT

FOOD SURVEY

This questionnaire forms part of a study being carried out by The Wythenshawe Transplant Centre.

If you have any queries, please telephone

Tim Entwistle: 0161 291 2281 or 07591937476

THANK YOU FOR YOUR COOPERATION

Date of completion: ______________________________
WE WOULD LIKE TO ASK YOU WHICH FOODS YOU EAT, AND HOW MUCH YOU EAT OF EACH.

On the next page you will see a list of foods with an amount written next to each food. For each food we would like you to indicate with a tick how often, on average, you have eaten the given amount over the last six months. This may vary from never, to four or more times as much as the given amount per day.

To help get you started, here are some examples of what we mean. If you can take a few minutes to work through these examples, you will quickly get the idea. The most important point is to answer the questions honestly, that way we gather all our information as precisely as possible.

EXAMPLE 1: How often do you drink the equivalent of 250 ml (8oz or 1 cup) of whole milk?

If, on average, you drink an amount equivalent to a 250 ml glass of whole milk every day (including milk you use on cereal, or in tea or coffee), you would place a tick in the 1 per day column, like this:

<table>
<thead>
<tr>
<th>Number of times used this amount over last 6 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>N</td>
</tr>
</tbody>
</table>

DAIRY & RELATED FOODS

Whole Milk 250ml glass (1/2 pint)

√

If you drink twice this amount, that is a total of about two 250ml glasses of whole milk every day, you would place a tick in the 2-3 per day column, like this:

<table>
<thead>
<tr>
<th>Number of times used this amount over last 6 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>N</td>
</tr>
</tbody>
</table>

OTHER VEGETABLES

Green Beans 1/2 cup

√

If you eat 1 cup of green beans a week, on average, this is the same as eating 1/2 cup of green beans 2 times a week, so you would place a tick in the 2-4 per week column, like this:

<table>
<thead>
<tr>
<th>Number of times used this amount over last 6 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>N</td>
</tr>
</tbody>
</table>

EXAMPLE 2: How often do you eat 1/2 cup of green beans?

If, on average, you eat 1/2 cup of green beans every 2 weeks, (= 1 cup green beans every 4 weeks) you would put a tick in the ‘1-3 per month’ column, like this:

<table>
<thead>
<tr>
<th>Number of times used this amount over last 6 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>N</td>
</tr>
</tbody>
</table>

If there are any foods that you occasionally or rarely eat, please place a tick in the LESS THAN 1 PER MONTH column. If there are any foods that you never eat, please place a tick in the NEVER column. Do not leave any column blank.
Q 1 Now, please look at the list of foods below. For each food listed indicate with a tick how often, on average, you have eaten this food, in the given amount, during the last 6 months. Please try to think carefully about each food, and try not to leave any blank lines.

<table>
<thead>
<tr>
<th>DAIRY &amp; RELATED FOODS</th>
<th>Number of times used this amount over last 6 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foods</td>
<td>Amount</td>
</tr>
</tbody>
</table>
| Ne
ever             | Less than 1 per m’th | 1-3 per m’th | 1 per week | 2-4 per week | 5-6 per week | 1 per day | 2-3 per day | 4+ per day |
| Skimmed milk (0.5% fat) | 250ml glass (1/2 pint) | | | | | | | |
| Semi skimmed milk (2% fat) | 250ml glass (1/2 pint) | | | | | | | |
| Whole milk            | 250ml glass (1/2 pint) | | | | | | | |
| Flavoured milk (e.g. Nesquik) | 250ml glass (1/2 pint) | | | | | | | |
| Soymilk (plain/flavoured) | 250ml glass (1/2 pint) | | | | | | | |
| Cream or sour cream   | 1 tablespoon                   | | | | | | | |
| Custard               | ½ cup (125ml or 4oz)           | | | | | | | |
| Ice cream             | ½ cup (125ml or 4oz)           | | | | | | | |
| Yoghurt, flavoured/plain | 1 small tub (200g)        | | | | | | | |
| Yoghurt, low fat, flav/plain | 1 small tub (200g)    | | | | | | | |
| Yoghurt, Greek, regular/low fat | 1 small tub (200g) | | | | | | | |
| Cottage or ricotta cheese | ½ cup (125ml or 4oz) | | | | | | | |
| Other low fat cheese  | 1 slice  (30g or 1oz)          | | | | | | | |
| Regular cheese (e.g. cheddar, brie, cream cheese) | 1 slice feta, (30g or 1oz) | | | | | | | |
| Margarine, spread on bread or food (Exclude use in cooking) | 1 teaspoon | | | | | | | |
| Butter, spread on bread or food (Exclude use in cooking) | 1 teaspoon | | | | | | | |
| Olive oil, added to bread or food (Exclude use in cooking) | 1 teaspoon | | | | | | | |
| What brand(s) and type(s) of margarine did you use most often for spreading on bread, vegetables or other foods? (Exclude use in cooking) (Please specify brand and type e.g. Flora, Benecol, I Can’t Believe Its Not Butter) | | | | | | | | |
| What brand(s) and type(s) of butter did you use most often for spreading on bread, vegetables or other foods? (Exclude use in cooking) (Please specify brand and type e.g. Lurpak, Country Life) | | | | | | | | |
Q2

Do you usually add butter or margarine to your bread? □ Yes □ No

Do you usually add butter or margarine to your vegetables or cooked foods? □ Yes □ No

Do you usually add Olive oil to your vegetables or cooked foods? □ Yes □ No

Q3

Number of times used this amount over the last 6 mths

<table>
<thead>
<tr>
<th>FRUITS</th>
<th>Number of times used this amount over the last 6 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Please indicate how often on average you eat these fruits when they are in season.</td>
<td>N e v e r</td>
</tr>
<tr>
<td>Foods</td>
<td>Amount</td>
</tr>
<tr>
<td>Fresh peaches, apricots, plums or nectarines</td>
<td>1</td>
</tr>
<tr>
<td>Fresh grapes</td>
<td>small bunch (about 20)</td>
</tr>
<tr>
<td>Fresh strawberries</td>
<td>½ cup</td>
</tr>
<tr>
<td>Blueberries, blackberries (fresh or frozen)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Other berries, cherries (fresh or frozen)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Cantaloupe, galia, honeydew melon</td>
<td>1/4 melon</td>
</tr>
<tr>
<td>Watermelon</td>
<td>1 slice</td>
</tr>
<tr>
<td>Fresh mangoes</td>
<td>1 mango</td>
</tr>
<tr>
<td>Fresh pineapple</td>
<td>1 slice</td>
</tr>
<tr>
<td>Avocado</td>
<td>1/2 avocado</td>
</tr>
<tr>
<td>Fresh apple or pear</td>
<td>1</td>
</tr>
<tr>
<td>Fresh orange, mandarin, tangerine</td>
<td>1</td>
</tr>
<tr>
<td>Fresh banana</td>
<td>1 medium</td>
</tr>
<tr>
<td>Prunes</td>
<td>½ cup</td>
</tr>
<tr>
<td>Dried apricots</td>
<td>4 – 5 halves</td>
</tr>
<tr>
<td>Dried peaches</td>
<td>4 – 5 halves</td>
</tr>
<tr>
<td>Other dried fruits</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td>(e.g. sultanas, raisins, dates)</td>
<td></td>
</tr>
<tr>
<td>Canned apricots or peaches in syrup or juice</td>
<td>½ cup</td>
</tr>
<tr>
<td>(e.g. Delmonte, Natures Finest)</td>
<td></td>
</tr>
<tr>
<td>Foods</td>
<td>Amount</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Broccoli</td>
<td>½ cup</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>½ cup</td>
</tr>
<tr>
<td>Spinach</td>
<td>½ cup</td>
</tr>
<tr>
<td>Spring onions, shallots</td>
<td>1 medium</td>
</tr>
<tr>
<td>Garlic (fresh or puree)</td>
<td>2 cloves</td>
</tr>
<tr>
<td>Potato (boiled or mashed)</td>
<td>1 medium potato</td>
</tr>
<tr>
<td>Potato (baked)</td>
<td>½ cup, or 1 medium</td>
</tr>
<tr>
<td>Hot chips, potato wedges, hash browns etc</td>
<td>1 cup</td>
</tr>
<tr>
<td>Pumpkin / Butternut (boiled or mashed)</td>
<td>1 medium piece</td>
</tr>
<tr>
<td>Pumpkin / Butternut (baked)</td>
<td>½ cup, 1 medium piece</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>½ cup</td>
</tr>
<tr>
<td>Carrots</td>
<td>1 medium whole or ½ cup cooked</td>
</tr>
<tr>
<td>Turnip / parsnip / swede</td>
<td>½ cup</td>
</tr>
<tr>
<td>Peas</td>
<td>½ cup</td>
</tr>
<tr>
<td>Green beans</td>
<td>½ cup</td>
</tr>
<tr>
<td>Cabbage (green or white)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>3-5 fresh or frozen</td>
</tr>
<tr>
<td>Sweet corn</td>
<td>1 cob or ½ cup frozen or canned</td>
</tr>
<tr>
<td>Beetroot (boiled or pickled)</td>
<td>1 small or 4 slices</td>
</tr>
<tr>
<td>Beetroot (grated)</td>
<td>1 small</td>
</tr>
<tr>
<td>Aubergine, courgette or squash</td>
<td>½ cup</td>
</tr>
<tr>
<td>Mushrooms (all varieties)</td>
<td>6-7 small</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>1 medium</td>
</tr>
<tr>
<td>Lettuce</td>
<td>2 medium leaves</td>
</tr>
<tr>
<td>Salad greens (spinach, rocket, watercress)</td>
<td>1 cup</td>
</tr>
<tr>
<td>Coleslaw</td>
<td>½ cup</td>
</tr>
<tr>
<td>Celery</td>
<td>10cm (4 inch) stick</td>
</tr>
<tr>
<td>Fresh Herbs (basil, rosemary)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Q 5</td>
<td>Number of times used this amount over last 6 mths</td>
</tr>
<tr>
<td>-----</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td><strong>Legumes / Beans</strong> (Dried/soaked or canned) (Please indicate how often on average you eat these foods)</td>
<td></td>
</tr>
<tr>
<td>Foods</td>
<td>Amount</td>
</tr>
<tr>
<td>Baked beans</td>
<td>½ cup</td>
</tr>
<tr>
<td>Soybeans</td>
<td>½ cup</td>
</tr>
<tr>
<td>Tofu (soy protein)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Beans (butter, kidney, cannellini, borlotti, black-eye etc)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Lentils (all varieties)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Chickpeas</td>
<td>½ cup</td>
</tr>
<tr>
<td>Hummus (chickpea/garlic dip)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Bean sprouts</td>
<td>½ cup</td>
</tr>
<tr>
<td>Foods</td>
<td>Amount</td>
</tr>
<tr>
<td>---------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Beef, pork or lamb as main dish (e.g. steak, roast)</td>
<td>1 small steak or 3 slices</td>
</tr>
<tr>
<td>Beef, pork or lamb as mixed dish (e.g. stew, casserole, stir-fry)</td>
<td>½ cup (125g)</td>
</tr>
<tr>
<td>Ham/pork, beef or lamb in sandwich</td>
<td>1 slice</td>
</tr>
<tr>
<td>Chicken with skin</td>
<td>1 drumstick or 2 slices</td>
</tr>
<tr>
<td>Chicken without skin</td>
<td>1 drumstick or 2 slices</td>
</tr>
<tr>
<td>Sausages</td>
<td>2 thick or 3 thin</td>
</tr>
<tr>
<td>Beef burger</td>
<td>1</td>
</tr>
<tr>
<td>Minced meat in tomato sauce or pasta sauce (e.g. Bolognase sauce)</td>
<td>1 cup</td>
</tr>
<tr>
<td>Other minced meat dishes</td>
<td>1 cup</td>
</tr>
<tr>
<td>Bacon</td>
<td>2 slices</td>
</tr>
<tr>
<td>Liver</td>
<td>1 slice (100g or 4oz)</td>
</tr>
<tr>
<td>Meat pie (e.g. pork pie)</td>
<td>1</td>
</tr>
<tr>
<td>Sausage roll</td>
<td>1</td>
</tr>
<tr>
<td>Processed meats (e.g. Corned beef, salami, chorizo, processed ham)</td>
<td>1 piece or slice</td>
</tr>
<tr>
<td>Frankfurt</td>
<td>1 large or 3 small</td>
</tr>
<tr>
<td>Boiled or poached egg</td>
<td>1</td>
</tr>
<tr>
<td>Fried egg</td>
<td>1</td>
</tr>
<tr>
<td>Scrambled egg or omelette</td>
<td>1 (with 1 egg)</td>
</tr>
<tr>
<td>Oily fish (e.g. mackerel, sardines, herring, trout, salmon)</td>
<td>125g serving</td>
</tr>
<tr>
<td>White fish (e.g. haddock, cod, monkfish), 125g serving and other fish (e.g. tuna fish)</td>
<td></td>
</tr>
<tr>
<td>Fish cakes or seafood sticks</td>
<td>1</td>
</tr>
<tr>
<td>Shellfish (e.g. prawns, mussels, cockles) ½ cup (125g) Crab, scallops</td>
<td></td>
</tr>
<tr>
<td>Soy based meat substitutes (e.g. Tofu, Cauldron products)</td>
<td>½ cup (125g)</td>
</tr>
<tr>
<td>Other meat substitutes (e.g. Quorn)</td>
<td>1 veggie burger (125g)</td>
</tr>
<tr>
<td>Q 7</td>
<td>Number of times used this amount over last 6 mths</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td><strong>BREAD, CEREALS, STARCHES</strong></td>
<td></td>
</tr>
<tr>
<td>Foods</td>
<td>Amount</td>
</tr>
<tr>
<td>Cold breakfast cereal</td>
<td>1 cup</td>
</tr>
<tr>
<td><strong>What brand(s) and type(s) of cold breakfast cereal do you use most often</strong> (Please specify brand and type e.g. Kellogg’s Special K, Nestle Shreddies, Weetabix)</td>
<td></td>
</tr>
<tr>
<td>Porridge oats (e.g. Quaker)</td>
<td>1 cup</td>
</tr>
<tr>
<td>Muesli</td>
<td>1 cup</td>
</tr>
<tr>
<td>White bread or toast</td>
<td>1 slice</td>
</tr>
<tr>
<td>Wholemeal/mixed grain/ rye bread or toast</td>
<td>1 slice</td>
</tr>
<tr>
<td>Soy and linseed bread or toast</td>
<td>1 slice</td>
</tr>
<tr>
<td>Scone or Crumpet</td>
<td>1 scone or 1 crumpet</td>
</tr>
<tr>
<td>Brown rice</td>
<td>1 cup (cooked)</td>
</tr>
<tr>
<td>White rice</td>
<td>1 cup (cooked)</td>
</tr>
<tr>
<td>White pasta e.g. macaroni, spaghetti, noodles, etc.</td>
<td>1 cup</td>
</tr>
<tr>
<td>Brown/Wholemeal pasta, noodles etc</td>
<td>1 cup</td>
</tr>
<tr>
<td>Other grains (e.g. couscous, barley, polenta)</td>
<td>1 cup</td>
</tr>
<tr>
<td>Crispbread, crackers (e.g. 1 Ryvita, Jacobs)</td>
<td>1</td>
</tr>
<tr>
<td>Cereal or Muesli bars (e.g. Kellogg’s, Alpen)</td>
<td>1</td>
</tr>
<tr>
<td>Oat cakes</td>
<td>1</td>
</tr>
<tr>
<td>Food</td>
<td>Amount</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>N ever</td>
</tr>
<tr>
<td>Beer (ordinary or strong)</td>
<td>1 bottle (330ml)</td>
</tr>
<tr>
<td>Beer (low alcohol)</td>
<td>1 bottle (330ml)</td>
</tr>
<tr>
<td>Cider</td>
<td>1 bottle (330ml)</td>
</tr>
<tr>
<td>Red Wine</td>
<td>1 glass (125 ml)</td>
</tr>
<tr>
<td>White Wine or Champagne</td>
<td>1 glass (125 ml)</td>
</tr>
<tr>
<td>Sherry or Port</td>
<td>1 small glass (60 ml)</td>
</tr>
<tr>
<td>Cream liqueurs</td>
<td>1 small glass (60 ml)</td>
</tr>
<tr>
<td>(e.g. Baileys)</td>
<td></td>
</tr>
<tr>
<td>Other liqueurs</td>
<td>1 small glass (60 ml)</td>
</tr>
<tr>
<td>(e.g Cointreau, Tia Maria, Kahlua, Amaretto)</td>
<td></td>
</tr>
<tr>
<td>Spirits</td>
<td>1 drink or 1 nip (30 ml)</td>
</tr>
<tr>
<td>(e.g. whiskey)</td>
<td></td>
</tr>
<tr>
<td>Pre mix drinks</td>
<td>1 can or 1 bottle</td>
</tr>
<tr>
<td>(e.g. Smirnoff Ice, VK, Bacardi Breezer)</td>
<td></td>
</tr>
<tr>
<td>BEVERAGES (WITHOUT ALCOHOL)</td>
<td>Number of times used this amount over last 6 mths</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Neve r</td>
</tr>
<tr>
<td>Amount</td>
<td></td>
</tr>
<tr>
<td>Water tap or bottled</td>
<td>(250 ml or ½ pint)</td>
</tr>
<tr>
<td>(Plain or sparkling)</td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td>1 small glass (125 ml ¼ pint)</td>
</tr>
<tr>
<td>Pineapple juice</td>
<td>1 small glass (125 ml ¼ pint)</td>
</tr>
<tr>
<td>Grape juice</td>
<td>1 small glass (125 ml ¼ pint)</td>
</tr>
<tr>
<td>Tomato juice or vegetable</td>
<td>1 small glass (125 ml ¼ pint)</td>
</tr>
<tr>
<td>juice</td>
<td></td>
</tr>
<tr>
<td>Carrot juice</td>
<td>1 small glass (125 ml ¼ pint)</td>
</tr>
<tr>
<td>Other fruit juice</td>
<td>1 small glass (125 ml ¼ pint)</td>
</tr>
<tr>
<td>Cola – regular (Coke, Pepsi)</td>
<td>1 can (330ml)</td>
</tr>
<tr>
<td>Cola - low calorie (e.g. Diet Coke)</td>
<td>1 can (330ml)</td>
</tr>
<tr>
<td>Cola - decaffeinated</td>
<td>1 can (330ml)</td>
</tr>
<tr>
<td>(e.g. Decaf Diet Coke)</td>
<td></td>
</tr>
<tr>
<td>Other soft drink (e.g. Sprite, Tango)</td>
<td>1 can (330ml)</td>
</tr>
<tr>
<td>Other low calorie soft drink</td>
<td>1 can (330ml)</td>
</tr>
<tr>
<td>(e.g. Diet Sprite)</td>
<td></td>
</tr>
<tr>
<td>Cordial (e.g. Robinsons)</td>
<td>1 glass (250ml or ½ pint)</td>
</tr>
<tr>
<td>Sport drink (e.g. Lucozade)</td>
<td>1 bottle (380ml)</td>
</tr>
<tr>
<td>Coffee</td>
<td>1 cup (1/2 mug*)</td>
</tr>
<tr>
<td>Decaffeinated Coffee</td>
<td>1 cup (1/2 mug*)</td>
</tr>
<tr>
<td>Tea (not herbal or green tea)</td>
<td>1 cup (1/2 mug*)</td>
</tr>
<tr>
<td>Green tea</td>
<td>1 cup (1/2 mug*)</td>
</tr>
<tr>
<td>Herbal tea</td>
<td>1 cup (1/2 mug*)</td>
</tr>
<tr>
<td>(e.g. chamomile, mint, fennel)</td>
<td></td>
</tr>
<tr>
<td>SWEETS, BAKED GOODS &amp; SNACKS</td>
<td>Number of times used this amount over last 6 mths</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td><strong>Foods</strong></td>
<td><strong>Amount</strong></td>
</tr>
<tr>
<td>Cake</td>
<td>1 slice</td>
</tr>
<tr>
<td>Tart or sweet pie</td>
<td>1 slice</td>
</tr>
<tr>
<td>Pastry, Pavlova, Cheesecake, etc</td>
<td>1 slice</td>
</tr>
<tr>
<td>Sweet roll (iced finger), bun etc</td>
<td>1 slice</td>
</tr>
<tr>
<td>Plain sweet biscuits (e.g. Nice, rich tea)</td>
<td>1 slice</td>
</tr>
<tr>
<td>Fancy biscuits, commercial (e.g. Kit Kat)</td>
<td>1 slice</td>
</tr>
<tr>
<td>Chocolate bar or packet 1 small bar/packet (50g) (e.g. M&amp;Ms, Cadbury Dairy Milk)</td>
<td>1 slice</td>
</tr>
<tr>
<td>Dark chocolate 1 – 2 squares or 1 bar (e.g. Lindt Excellence, Bourneville)</td>
<td>1 slice</td>
</tr>
<tr>
<td>Lollies without chocolate</td>
<td>3-5</td>
</tr>
<tr>
<td>Jam, marmalade, syrup or honey</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td>Marmite or Vegemite</td>
<td>1 teaspoon</td>
</tr>
<tr>
<td>Nuts (including salted)</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td>Seeds (e.g. pumpkin, sunflower)</td>
<td>1 tablespoon</td>
</tr>
<tr>
<td>Popcorn (salted or sweet)</td>
<td>1 cup</td>
</tr>
<tr>
<td>Crisps 1 small bag (30g) (e.g. Walker’s, Kettle Chips, Wotsits)</td>
<td>1 slice</td>
</tr>
</tbody>
</table>
**OTHER FOODS**

<table>
<thead>
<tr>
<th>Foods</th>
<th>Amount</th>
<th>Never</th>
<th>Less than 1 per m'th</th>
<th>1-3 per m'th</th>
<th>1 per week</th>
<th>2-4 per week</th>
<th>5-6 per week</th>
<th>1 per day</th>
<th>2-3 per day</th>
<th>4+ per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pizza</td>
<td>2 slices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olives (green or black)</td>
<td>1/3 cup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pickled vegetables (e.g. onions, gherkins)</td>
<td>1/3 cup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creamy soup</td>
<td>1 cup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil and vinegar dressing (e.g. French)</td>
<td>1 tablespoon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayonnaise or other creamy salad dressing</td>
<td>1 tablespoon salad dressing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat free mayonnaise free salad dressing</td>
<td>1 tablespoon or fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato sauce/ketchup</td>
<td>1 tablespoon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream based pasta sauces (e.g. Creamy Carbonara)</td>
<td>½ cup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q 11  On average (not counting juices) how many serves of fruit do you usually eat per day? (Please tick one)
(a serve = 1 medium piece or two small pieces of fruit or 1 cup of diced pieces)

1. [ ] None
2. [ ] 1 serve
3. [ ] Between ½ and 1 serve
4. [ ] Rarely (less than a ½ serve)
5. [ ] 2 serves
6. [ ] 3 serves
7. [ ] 4 or more serves

Q 12  On average, how many serves of vegetables do you usually eat per day? (Please tick one)
(a serve = ½ cup vegetables or 1 cup of salad vegetables)

1. [ ] None
2. [ ] 1 serve
3. [ ] Between ½ and 1 serve
4. [ ] Rarely (less than a ½ serve)
5. [ ] 2 serves
6. [ ] 3 serves
7. [ ] 4 or more serves

Q 13  On average, how many servings of red meat do you usually eat per day? (Please tick one) (by meat we mean beef, lamb, liver, kidney, pork, ham); (a serve = 1 small steak, 3 slices or ½ cup)

1. [ ] None
2. [ ] 1 serve
3. [ ] Between ½ and 1 serve
4. [ ] Rarely (less than a ½ serve)
5. [ ] 2 serves
6. [ ] 3 serves
7. [ ] 4 or more serves
**How often do you eat fish?** (one average serving = approximately 125g or 1 small fillet) *(Please tick one)*

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>Less than 1 per month</td>
<td>5</td>
</tr>
<tr>
<td>1-3 times per month</td>
<td>6</td>
</tr>
<tr>
<td>Once per week</td>
<td>7</td>
</tr>
<tr>
<td>2-4 times per week</td>
<td>8</td>
</tr>
<tr>
<td>5-6 times per week</td>
<td>9</td>
</tr>
<tr>
<td>Once per day</td>
<td>10</td>
</tr>
<tr>
<td>More than once per day</td>
<td>11</td>
</tr>
</tbody>
</table>

**How often do you eat shellfish?** (i.e. prawns, mussels, crab) *(Please tick one)*

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>Less than 1 per month</td>
<td>5</td>
</tr>
<tr>
<td>1-3 times per month</td>
<td>6</td>
</tr>
<tr>
<td>Once per week</td>
<td>7</td>
</tr>
<tr>
<td>2-4 times per week</td>
<td>8</td>
</tr>
<tr>
<td>5-6 times per week</td>
<td>9</td>
</tr>
<tr>
<td>Once per day</td>
<td>10</td>
</tr>
<tr>
<td>More than once per day</td>
<td>11</td>
</tr>
</tbody>
</table>

**How often do you eat plain nuts?** (i.e. walnut, almonds, hazelnuts) *(Please tick one)* (one average serving = approximately 28g or 1 handful, or 14 shelled walnut halves, or 24 almonds)

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>Less than 1 per month</td>
<td>5</td>
</tr>
<tr>
<td>1-3 times per month</td>
<td>6</td>
</tr>
<tr>
<td>Once per week</td>
<td>7</td>
</tr>
<tr>
<td>2-4 times per week</td>
<td>8</td>
</tr>
<tr>
<td>5-6 times per week</td>
<td>9</td>
</tr>
<tr>
<td>Once per day</td>
<td>10</td>
</tr>
<tr>
<td>More than once per day</td>
<td>11</td>
</tr>
</tbody>
</table>

**Q 12 How many teaspoons of sugar (altogether) do you add to your food and drink each day?** *(Include sugar added to your tea, coffee, cereal, fruit etc.)*

Total ____ tps

**Q 13 What type of cooking oil is used most often in your home?**

Please specify brand and type (e.g. Crisp & Dry, Olivio, or Olive oil) ________________________________

**Q 14 What kind of fat is used most often in your home for frying or roasting meat or vegetables?** *(Please tick one)*

<table>
<thead>
<tr>
<th>Fat Type</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butter</td>
<td>1</td>
</tr>
<tr>
<td>Lard</td>
<td>2</td>
</tr>
<tr>
<td>Cooking (solid) margarine</td>
<td>3</td>
</tr>
<tr>
<td>Polyunsaturated margarine</td>
<td>4</td>
</tr>
<tr>
<td>Extra or Virgin Olive oil</td>
<td>5</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>6</td>
</tr>
<tr>
<td>Other, please specify</td>
<td>7</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
</tr>
</tbody>
</table>
Q 15  How often do you eat food that is fried at home? (Include any foods cooked in a pan or on a hot plate e.g. pan frying or dry frying) (Please tick one)

☐ Less than once per week ☐ Daily
☐ 1-3 times per week ☐ 2 or more times per day
☐ 4-6 times per week ☐ Never

Q 16  How often do you eat take-away that is fried? (e.g. hot chips, battered foods, fried chicken or fried fish) (Please tick one)

☐ Less than once per week ☐ Daily
☐ 1-3 times per week ☐ 2 or more times per day
☐ 4-6 times per week ☐ Never

Q 17  Do you take fish oil capsules (or liquid)? (Please tick one)

☐ Less than once per week ☐ Daily
☐ 1-3 times per week ☐ 2 or more times per day
☐ 4-6 times per week ☐ Never

Please note: If you need help completing this page, tick this box ☐ and we will contact you.

Thank you for completing this questionnaire
Appendix E. Mediterranean diet 14-point adherence questionnaire

Place a tick in right hand box if your answer is YES. If NO – please leave blank

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Do you use extra virgin olive oil as main culinary/cooking fat/oil?</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>How much extra virgin olive oil do you eat in a given day (including oil used for cooking, salads, out-of-home meals etc.)?</td>
<td>4 tbsp or more</td>
</tr>
<tr>
<td>3</td>
<td>How many vegetable servings do you eat per day? (1 serving = 200 g or side dish as a half serving)</td>
<td>2 or more (cooked) or 1 or more (raw or as salad)</td>
</tr>
<tr>
<td>4</td>
<td>How many fruit units (including natural fruit juices) do you eat per day?</td>
<td>3 or more</td>
</tr>
<tr>
<td>5</td>
<td>How many servings of red meat, beef-burger or meat products (ham, sausage, bacon etc.) do you eat per week?</td>
<td>1 or less</td>
</tr>
<tr>
<td>6</td>
<td>How many servings of butter, margarine or cream do you eat per day? (1 serving: 12 g or 1 tablespoon)</td>
<td>1 or less</td>
</tr>
<tr>
<td>7</td>
<td>How many sweetened and/or carbonated beverages do you drink per day?</td>
<td>Less than 1</td>
</tr>
<tr>
<td>8</td>
<td>How much red wine do you drink per week?</td>
<td>7 glasses or more</td>
</tr>
<tr>
<td>9</td>
<td>How many servings of legumes/pulses do you eat per week? (1 serving: 150 g or ½ cup in dry form)</td>
<td>3 or more</td>
</tr>
<tr>
<td>10</td>
<td>How many servings of fish or shellfish do you eat per week? (1 serving 100–150 g of fish (size of deck of cards) or 4–5 units or 200 g of shellfish)</td>
<td>3 or more</td>
</tr>
<tr>
<td>11</td>
<td>How many times per week do you eat desserts or pastries (not homemade), such as biscuits, cookies, cakes, custard etc?</td>
<td>Less than 3</td>
</tr>
<tr>
<td>12</td>
<td>How many servings of unsalted nuts (including peanuts) do you eat per week? (1 serving = 30 g or 1 small handful)</td>
<td>3 or more</td>
</tr>
<tr>
<td>13</td>
<td>Do you prefer to eat chicken, turkey or lean game instead of pork, beef-burger, sausage or bacon?</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>How many times per week do you eat a self-made sauce (sorrito) prepared with tomato, garlic, onion or leeks - simmered in olive oil. Used as a garnish to season vegetable, pasta or rice dishes?</td>
<td>2 or more</td>
</tr>
</tbody>
</table>
## Appendix F. Low-fat diet 9-point adherence questionnaire

**Please circle your answer to each question (circle 1 answer per question)**

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>0-1</th>
<th>2-4</th>
<th>5+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>On average, how many tablespoons of vegetable oil (e.g. sunflower, olive, sesame, rapeseed oil) do you consume in a given day for frying, salads &amp; out of house meals etc?</td>
<td>0-1</td>
<td>2-4</td>
<td>5+</td>
</tr>
<tr>
<td>2</td>
<td>Do you remove visible fat (or skin) from chicken, duck, pork, lamb or beef before cooking; and the fat from soups, broths and cooked meat dishes before eating?</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>How many servings of non-lean meats, hamburger, commercial minced meat, sausage, cold meat, cured ham, bacon, salami or offal do you consume per week? (meat serving = 100g or salami or bacon 30g)</td>
<td>0-1</td>
<td>2-4</td>
<td>5+</td>
</tr>
<tr>
<td>4</td>
<td>How many servings of butter, margarine, lard, mayonnaise, milk-cream or milk-based ice cream do you consume per week? (spread fat serving = 12g or ice cream = 100g)</td>
<td>0-1</td>
<td>2-4</td>
<td>5+</td>
</tr>
<tr>
<td>5</td>
<td>Do you exclusively consume low-fat dairy products?</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>How many times per week do you eat fried foods e.g. chips/French fries or fried chicken?</td>
<td>0-1</td>
<td>2-4</td>
<td>5+</td>
</tr>
<tr>
<td>7</td>
<td>How many times per week do you consume fatty fish or fish or seafood canned in oil?</td>
<td>0-1</td>
<td>2-4</td>
<td>5+</td>
</tr>
<tr>
<td>8</td>
<td>How many servings of commercial sweets or industrial bakery products (not homemade), such as cakes, cookies, biscuits or custard do you consume per week? (cake serving = 80g or 6 biscuits 40g)</td>
<td>0-1</td>
<td>2-4</td>
<td>5+</td>
</tr>
<tr>
<td>9</td>
<td>How many times per week do you consume nuts (including peanuts), potato, corn or wheat crisps or commercial snacks?</td>
<td>0-1</td>
<td>2-4</td>
<td>5+</td>
</tr>
</tbody>
</table>
Appendix G. Household member questionnaire

Dear (Name)

As you may be aware, the recent dietary study that you & (Name) were involved in has reached its 12-month end point. We hope this study has helped you both to make healthy eating choices. We would like to assess whether you observed any changes in either (Name) health & wellbeing, or very importantly, in your own. Therefore, it would be greatly appreciated if you could take the time to answer the questions below so we can learn your opinion. You will also find an “additional comments” box for any further thoughts &/or feelings you may like to add. Thank you for your kind co-operation.

Please tick the box you feel most appropriate:

<table>
<thead>
<tr>
<th></th>
<th>Much Worse</th>
<th>Slightly Worse</th>
<th>No Change</th>
<th>Slightly Better</th>
<th>Much Better</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Has your partner’s health and wellbeing changed since taking part in the dietary study?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Do you feel being associated with this study has changed your own health and wellbeing?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Has your own weight changed in the last 12 months?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Have your own energy levels changed in the last 12 months?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Has this study helped you to eat a healthier diet?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Did following the study diet increase the cost of your weekly food bill?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Overall, did you find shopping for food items associated with the study diet more difficult than following your previous dietary habits?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Any additional comments?
Appendix H. Training session feedback form

<table>
<thead>
<tr>
<th></th>
<th>Poor</th>
<th>Fair</th>
<th>Good</th>
<th>Very good</th>
<th>Outstanding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to the day, how well informed did you feel?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Was the background information to the study presented in a manner that was understandable?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Did Tim make good use of his time?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Was Matt’s cooking demonstration presented / structured in a useful manner?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Did Matt make good use of his time?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Has this study helped change your understanding of food for the better?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Was the printed information you received sufficient enough to keep you on track?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Was this event what you expected?</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Overall, how satisfied were you with this event</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

Additional comments
Appendix I. Contents of Mediterranean diet information booklet
Life-style advice for healthy change

Some tips for changing your life style

A. Do not skip any of your main meals
   Three main courses and 1-2 snacks each day

B. Have only one plate-serving
   You can use a smaller plate to reduce the amount to eat

C. Eat plenty of vegetables and 2-4 servings of fruits/berries each day.
   Choose a variety of different coloured fruit/berries

D. Limit your intake of white bread and sweets to once a week,
   if you cannot do without!
   Substitute with whole-grain and slow carbohydrates instead

E. Always use at least five different ingredients in your main meals
   That way you will eat more varied.

F. Perform physical exercise at least 20 – 30 min each day
   Walk, jog, bicycle, swim, or whatever you enjoy

G. Eat slowly & chew properly; enjoy your delicious food.
   Put your knife & fork down in between each mouthful. This will reduce your hunger

H. Be kind to yourself
   Change can take time; be mindful, healthy foods & thoughts positively alter your health
**Healthy Shopping Tips**

- Keep a shopping or tick list handy in the kitchen/home, that way you can jot things down through the week as they pop into your mind
- If you can find the time or feel inclined, make a meal planner, that way you can buy in the ingredients you need on your next shopping trip
- Never go shopping on an empty stomach; feeling hungry will tempt you to impulse buy – this will tend to be something unhealthy
- Aim to buy fresh whenever possible; in general, fresher produce will taste better & contain more nutrients
- Aim to shop around the edge of the supermarket first; it tends to be where fresh foods like vegetables, fruit, fish, poultry & dairy are located
- When buying fruit & vegetables, think of a rainbow. Bright colours reflect the different vitamins, minerals, & antioxidants contained in each fruit or vegetable
- Be adventurous; aim to try a new fruit or vegetable each week, or better still, try seasonal produce
- Although you should aim to purchase fresh vegetables when possible, frozen varieties can be a great addition to a meal & ensure you always have something at hand. Garden peas are a great example as they are said to contain the same amount of nutrients as fresh (if not more). Other examples are broccoli, green beans, Brussels sprouts, cauliflower & carrots.
- Avoid the centre aisles, at least until you do the bulk of your shopping. It’s generally where the processed foods lurk; those full of sugar, salt, trans-fats & preservatives that extend shelf life
- Read the label. Although most unprocessed foods do not generally need one, anything else that is boxed or packaged deserves a quick check over
- It is worth remembering that if you struggle to recognise, pronounce or spell the contents on a label, would you want to eat it?
- Be aware of labels stating ‘natural ingredients’, they can be misleading, have a read first
- Check the label for sugar. Refined sugar can take many names; cane, brown, date, grape or beet sugar, glucose, sucrose, maltose, dextrose, fructose, maltodextrin, dextran, sorbitol, corn syrup, high fructose corn syrup, corn sugar, carob & sorghum syrup, fruit juice, fruit juice concentrate, barley malt, caramel
- To help you (or your family) get used to whole grains, you could start out with whole-wheat blends before changing to 100% whole-wheat breads & pasta etc.
- Buy in ‘staple’ foods, such as tinned tomatoes, butter beans, fish, or onions, garlic, pasta & rice etc. That way you will always have basic ingredients at home to make a tasty meal
- Think about buying basic foods in bigger bags (or bulk) if you use them regularly. Dried foods such as rice, lentils, seeds, nuts etc. It often works out far cheaper
- You may want to pack a freezer bag or pop one in the car to keep fish etc cool on your return home
**Fruit & Vegetable Preparation**

A really important part of the Mediterranean diet is eating a variety of fresh fruits & vegetables. Ideally they will be prepared in a manner that retains as many vitamins & minerals as possible. This may require you to make changes in the way you prepare & cook foods. Eating more of your vegetables raw or crunchier can also help you feel fuller with fewer calories; useful if you want to lose weight.

**Washing:**

Depending on the type of fruit &/or vegetable you are preparing, some will first require a good scrub; others can be rinsed & peeled/de-skinned as necessary. As with all the foods you eat, try to remember to minimise your infection risk by pre-washing & preparing foods on clean surfaces. Washing your produce also helps reduce surface pesticides & chemicals that may have been sprayed onto them when growing. Certain produce such as pears, pineapple, avocado etc may need to ripen before eating.

- Washing your hands before preparing food will reduce the chance of catching a bug: especially if it will be eaten raw or stored in the fridge
- Wash your fruit & vegetables under a cold running tap
- Ensure your knives/utensils, chopping board & work surfaces are thoroughly clean
- Much of the produce we buy has generally been pre-cleaned, but still rinse before using
- Any soil on carrot or potatoes etc. can be easily removed with a nail brush or vegetable scrubber.
- If packaging says ‘ready-to-eat’ you must still wash before eating
- Try to avoid eating food that is damaged, bruised or over-ripe
- Do not pre-wash tomatoes until you start to prepare your meal as it can affect their quality

**Preparation & Cooking Methods:**

Preparing fruit & vegetables in differing ways may enable you to try foods you may normally not eat. Depending on how you prepare your vegetables, you can change the taste & texture significantly. Some people prefer eating raw carrot whole or sliced into strips (Julienned), others grated, which makes them juicier. Roasting or steaming carrots can make them taste sweeter & retain lots of goodness - it can also make them easier to digest. You may want to make a carrot soup or add them to a stew/casserole.

- Making a meal plan in advance can help motivate & ensure you have the ingredients you require rather than having to nip out
- Remember to marinate or defrost foods in advance, & if required, pre-warm your oven
- Decide what to cook first. You may want to start some brown rice before preparing other foods to go with the dish you are planning
- Remember, a sharp knife is essential in the kitchen - it will make chopping easier & safer!
- When preparing a meal with different vegetables e.g. stir-fry; cut the vegetables to similar sizes to ensure they cook at a similar rate

**Raw**

- Preparation will change taste & texture - be adventurous
- Try slicing carrots, parsnips or courgette with a potato peeler; it will quickly slice them into wafer thin strips to add to a meal or salad.

**Steam or boil**

- Steaming is a quick & healthy way to cook vegetables. Because they have no direct contact with water, it helps retain beneficial nutrients & leaves a crunchier texture
Roast or bake
- Roasting or baking increases taste & sweetness, try parsnips, carrots, beetroot /or sweet potato with a drizzle of extra-virgin olive oil. Adding crushed garlic or pieces of onion adds lovely flavour
- Again, cut to similar sizes to ensure your vegetables cook at similar rates

Grill
- Try grilling sweet peppers or mushrooms with a drizzle of olive oil or add some low-fat cheese

Stir-fry or sauté
- A great way to quickly prepare a meal is to stir-fry your vegetables. Not only will it save time, but the slight browning will add taste & leave your vegetables with a crunchy texture
- Covering your vegetables & simmering (sautéing) enables them to steam cook with little or no oil. It is a great way to prepare onions & garlic for a sauce without burning, or for adding to other dishes. Tip* it helps to slice them thinly

Soup, stew or casserole
- These 3 cooking methods allow a huge range of meals to be prepared. In general they are inexpensive & meals can be bulked with pulses & whole-grains such as barley
- It is a useful way of using up vegetables that may have passed their best for eating raw & thorough cooking will kill any bacteria

Juicing
- Fresh juices are a really healthy way of preparing fruits & vegetables & a mix can work equally well. The addition of half a lemon or lime will add a lovely zing, as can a thumb nail sized piece of fresh ginger

Storage:
Depending on how you store your fruit & vegetables, you can change the taste & texture considerably. You can store most things in the refrigerator, though potatoes, tomatoes, bananas, lemons & limes just require storage in a cool dry area. Onions & garlic should also be stored in a similar place. Remember to place any fish or meat at the bottom of your refrigerator in case any liquid drips & contaminates your fruit & vegetables below.

- Store raw food (including vegetables) separate from ready-to-eat foods
- Any sealed bags should be snipped open (if not pre-washing) before storing
- If you have cooked too much or need to use food up without wasting something, you can always refrigerate it for a next day meal, or freeze it for future use
- Fruits or vegetables can be prepared & stored raw in the freezer for future use (but not lettuce or soft leaf herbs). Fruits or vegetables are best stored in chopped pieces, making defrosting & cooking quicker
- Herbs (& asparagus) can be stored in a glass of water similar to flowers to keep them fresher for longer
# Fruit & Vegetable storage chart

<table>
<thead>
<tr>
<th>Store in refrigerator</th>
<th>Store on worktop</th>
<th>Cool, dry place</th>
<th>Ripen on worktop, then refrigerate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Apples (storage &gt;7 days)</em></td>
<td><em>Apples (storage &lt; 7 days)</em></td>
<td>All squash’s including butternut squash</td>
<td><em>Avocados</em></td>
</tr>
<tr>
<td><em>Apricots</em></td>
<td><em>Bananas</em></td>
<td>Pumpkins</td>
<td><em>Nectarines</em></td>
</tr>
<tr>
<td><em>Cantaloupe</em></td>
<td><em>Tomatoes</em></td>
<td>Onions (away from potatoes)</td>
<td><em>Peaches</em></td>
</tr>
<tr>
<td><em>Figs</em></td>
<td>Basil</td>
<td>Potatoes (away from onions)</td>
<td><em>Pears</em></td>
</tr>
<tr>
<td><em>Honeydew</em></td>
<td>Cucumbers</td>
<td>Sweet potatoes</td>
<td><em>Plums</em></td>
</tr>
<tr>
<td>Artichokes</td>
<td>Aubergine/Eggplant</td>
<td>Yams</td>
<td></td>
</tr>
<tr>
<td>Asparagus</td>
<td>Garlic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beets</td>
<td>Ginger</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blackberries</td>
<td>Lemons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blueberries</td>
<td>Limes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli</td>
<td>Mangoes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>Oranges</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage</td>
<td>Clementine’s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrots</td>
<td>Satsuma’s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Tangerines</td>
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<td>Celery</td>
<td>Papayas</td>
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<tr>
<td>Cherries</td>
<td>Peppers</td>
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<td>Corn</td>
<td>Persimmon (Sharon fruit)</td>
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<td>Grapes</td>
<td>Pineapple</td>
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<td>Green beans</td>
<td>Plantains</td>
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<tr>
<td>Green onions</td>
<td>Pomegranates</td>
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<tr>
<td>Herbs (except basil)</td>
<td>Watermelon</td>
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<tr>
<td>Lima beans</td>
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<td><strong>Leafy vegetables</strong></td>
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<td>Leeks</td>
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<td>Lettuce</td>
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<td>Mushrooms</td>
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<td>Okra (ladies fingers)</td>
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<td>Peas</td>
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<td>Plums</td>
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<td>Radishes</td>
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<td>Raspberries</td>
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<td>Spinach</td>
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<td>Sprouts</td>
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<td>Strawberries</td>
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<td>Summer squash</td>
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<td>Yellow squash</td>
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<td></td>
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<tr>
<td>Courgette</td>
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*In ‘**bold italics**’ are the ethylene producing fruits that give off a natural odourless, harmless & tasteless gas. All fruit & vegetables release it, some more than others. Certain fruits & vegetables are sensitive to it & if stored close together will speed up the ripening process.

**Tip** - you can use this to your advantage (i.e. storing an unripe avocado next to a banana will speed ripening).
Fish & Shellfish Safety Tips

Part of your diet will include eating a range of fresh fish & shellfish, which contain healthy fats, protein, vitamins & minerals. In general, seafood is simple & quick to prepare, & if you have ever visited the Mediterranean, you will be aware that seafood is a common ingredient.

However, marine foods do carry a risk of bacterial infection if not stored or prepared properly; here are a few guidelines to ensure you minimise your risk of infection (especially shellfish).

When Purchasing:
- Aim to purchase seafood at the end of your shopping trip to retain a cool temperature
- Fresh fish should not smell overly ‘fishy’ & should be cold to the touch
- Try to purchase the freshest looking fish. Whole fish should have clear, rounded bright eyes, no slime & bright coloured gills
- When bagging food at the checkout, ask for a separate bag to keep fish products away from other foods
- Try & store seafood cool until you return home; you could always keep a small freezer bag handy for your return trip

At Home:
- Depending on fish type & quality, storage can vary greatly. Aim to use fish & shellfish within 1 – 2 days of purchase (if refrigerated)
- If freezing, store fish in the coldest part of your freezer
- If you purchase larger portions, you may want to portion it into smaller/multiple meals before freezing
- Plastic chopping boards are safer than wood when cleaning & sterilising. Ensure you clean surfaces & knives etc. after each use.
- Forgetting to clean surfaces before preparing other foods creates significant infection risk
- Defrost fish in the refrigerator; not at room temperature. Failing this, if still frozen, under a cold running tap
- Marinate seafood in the refrigerator, not at room temperature
- Refrigerate cooked seafood within 1 hour & aim to eat it the next day
  Never eat raw or improperly cooked fish & shellfish
- If smoked pre-cooked fish are purchased; ensure they are properly cooked if re-heated

Eating Out:
- As with all foods; if you suspect an unclean cooking environment, refrain from eating
- Ask that your fish be freshly prepared & properly cooked
- Shellfish carries the greatest risk; ask that it be freshly prepared & well-cooked e.g. paella
- Never eat pre-cooked fish/shellfish products served cold, you won’t know how long they have been left before serving (e.g. prawn salad)
- If smoked pre-cooked fish are purchased; ensure they are properly cooked if re-heated

Remember, this advice is in the interest of precaution. Do not let it deter you from trying a range of new seafood tastes & dishes. Enjoy.
Whole-grain & Preparation

What is a Whole-grain?
Whole-grains are seeds & cereals prepared as nature intended, that is, whole & intact. When eaten in their ‘whole’ form, examples include wheat, oats, brown rice, barley, quinoa (pronounced- Keen-wahl), spelt, sorghum & rye. When shopping, you can start to make simple changes by switching from refined white products to whole grain bread, pitta’s or crackers.

Unlike refined grains, whole grains are not stripped of their outer covering which means they retain a whole lot more fibre & vital nutrients, vitamins & minerals. On the other hand, refined grains & products have been stripped of their beneficial coatings. Outer (bran) & inner (germ) parts are discarded & the middle is used to produce foods such as white flour/bread. Unfortunately this means they lack most of their healthy nutrients & in the process lose taste. Whole-grains generally have a nuttier, fuller flavour & are slightly chewier than refined grains. As your taste changes, you may start to find those refined white products & grains become bland.

Better for your health
Carbohydrates (or energy) contained in whole grains are released at a slower rate than refined grains/products. This reduces blood sugar levels & releases energy in a more sustained manner, which can help you feel fuller for longer & improve your energy levels. Increased fibre content also helps slow food digestion & energy release, having the added effect of lowering blood cholesterol. Interestingly, people who regularly eat whole-grains have a lower risk of obesity & high cholesterol. Research shows 3 daily servings can help reduce the risk of heart disease, diabetes, stroke & certain cancers.

The next stage is learning which grains to purchase & how to prepare whole-grain dishes.

Cooking & Soaking Whole-grains
There are many ways you can incorporate whole-grains into your diet, simply by switching to products that contain them (e.g. breads, pasta or breakfast cereal) or changing from white to brown rice. Pasta dishes just need substituting for a whole-grain variety (such as whole-wheat, spelt or even buckwheat) & can be cooked in a similar manner & time. Brown rice on the other hand requires a longer cooking time unless pre-soaked. In some instances you may need to think ahead & plan your meals in advance or experiment with what works best. Soaking grains reduces cooking time & improves nutrient content & taste. Oats covered in a little water & soaked overnight cook in minutes & make a quick, tasty breakfast. Some grains benefit from the taste of ‘stock’ rather than water, or flavour can be improved by dry frying or toasting before cooking. Most grains cook in a similar manner to rice, or just need adding to soups, broths, stews or casseroles.
**Here are some basic guidelines:**
Cooking time will vary depending on the variety of grain & choice of pan.

1. Rinse grain & bring liquid/stock to the boil
2. Add grain, stir & bring back to boil
3. Cover your pan & reduce heat to a low setting (until water is absorbed). If water is absorbed & grains are still firm, you may want to add a touch more water, or drain excess if the reverse
4. Turn heat off & allow grains to rest for 5-10 minutes
5. If grains have stuck to the bottom of the pan, turn off heat & add a small amount of water, recover & let sit for a few minutes. The grains will lift off the base

**Hints & Tips:**
1. Pre-soak grains if you require them to cook quicker (& reduce energy bills)
2. Try cooking in larger batches & place in your refrigerator; they will keep fresh for a couple of days to add to salads or soups, or re-heat *thoroughly* in water/broth for a quick meal.
3. Ensure rice/grains are not left at room temperature for any length of time before refrigerating or freezing

<table>
<thead>
<tr>
<th><em>Please remember</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>• If re-heating rice, ensure it has been stored in the fridge for no longer than 1 day &amp; <strong>not</strong> at room temperature</td>
</tr>
<tr>
<td>• Keep rice in the fridge for <strong>no more than one day</strong> until reheating.</td>
</tr>
<tr>
<td>• When you re-heat your rice; always check that the dish is steaming hot all the way through.</td>
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</tbody>
</table>

4. Alternatively, you can make a larger batch & portion it up in freezer bags/containers & freeze to save time for future meals. You could do the same with leftovers if you have enough for another meal
5. **Grain Pilaf:** finely chop onion & garlic into small chunks (mushrooms too if you enjoy them) & gently fry in a little olive oil. Add in your grain & simmer together, stirring & coating. Add in vegetable or chicken broth/stock cube (quantities below) & cook until liquids becomes absorbed. It adds lots of flavour to your meal

Whole-grain cooking chart can be found overleaf
## Cooking times & quantity

<table>
<thead>
<tr>
<th>1 cup of grain</th>
<th>Add this amount of Water or broth</th>
<th>Bring to the boil then Simmer for:</th>
<th>Notes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranth</td>
<td>2-cups</td>
<td>20-25 minutes</td>
<td></td>
</tr>
<tr>
<td>Barley (hulled)</td>
<td>3-cups</td>
<td>1 – 1 &amp; ½ hours</td>
<td>Cooks slower than pearl barley</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>2-cups</td>
<td>15-20 minutes</td>
<td>Toasting first improves taste</td>
</tr>
<tr>
<td>Bulgur</td>
<td>2-cups</td>
<td>3-5 minutes</td>
<td>Let stand (covered) for 25-30 minutes</td>
</tr>
<tr>
<td>Couscous (whole-wheat)</td>
<td>2-cups</td>
<td>1-2 minutes</td>
<td>Leave to stand for 10 minutes</td>
</tr>
<tr>
<td>Farro</td>
<td>3-cups</td>
<td>1&amp;1/2 – 2 hours</td>
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<tr>
<td>Kamut (Khorasan wheat)</td>
<td>4-cups</td>
<td></td>
<td>Soak overnight – cook for 45-60 minutes</td>
</tr>
<tr>
<td>Millet (hulled)</td>
<td>2-cups</td>
<td>20-30 minutes</td>
<td></td>
</tr>
<tr>
<td>Quinoa (pronounced keen-wah)</td>
<td>2-cups</td>
<td>15-20 minutes</td>
<td>Rinse first. Toasting can add taste</td>
</tr>
</tbody>
</table>

### Oats
- 1-cup of water, then if you prefer, add milk or soy milk when cooking to bring to the desired consistency
- Soak overnight for a quick morning porridge (try adding in a few dried chopped fruits to add taste)

### Pasta (whole-wheat)
- 6-cups
- 8-12 minutes depending on size/type
- Serve “Al-Dente”

### Polenta
- 1-1/2 cups
- 3-5 minutes

### Rice (short grain)
- 2-cups
- 25-35 minutes
- Good for risotto & rice pudding

### Rice (long grain)
- 2-1/2 cups
- 25-35 minutes
- Good for pilaf & side dishes (quite fluffy)

### Rice (Basmati)
- 2-cups
- 30-40 minutes
- Long grain fragrant rice

### Rice (Jasmine)
- 2-cups
- 30-40 minutes
- Aromatic Thai long grain

### Rice (red)
- 2-cups
- 40-45 minutes
- Nutty, taste & texture

### Rice (wild)
- 3-4-cups
- 45-55 minutes
- Add to a salad or a handful in a risotto

### Rice (sweet)
- 1-1/2 cups
- 40-45 minutes
- Sticky Thai long grain

### Rice (risotto-1)
- 4-cups
- 40-60 minutes
- If cooking like other rice with lid on

### Rice (risotto-2)
- 4-5 cups
- 1-1/2 to 2 hours
- If adding liquid bit by bit

### Rye (berries/grain)
- 4-cups
- Soak overnight then cook 45-60 minutes
- Looks similar to wheat, but lower gluten level

### Spelt (berries/grain)
- 4-cups
- Soak overnight then cook 45-60 minutes
- Older wheat variety, lower in gluten

### Wheat (berries/grain)
- 2-1/2 cups
- Soak overnight then cook 45-60 minutes
- Chewy, nutty texture
Fats - Information

Fats are an essential part of our diet, yet the information about the various types may at times appear confusing. You read one thing in a newspaper, the next week it seems something on the news contradicts what you previously read. Below are a few simple points based on the main dietary fats we may encounter on a daily basis. A point worth remembering is that although fat intake differs significantly around the world, the longest lived populations tend to eat low levels of saturated fat, cook very little with frying methods & tend to include oils from fish or will add them to their diet uncooked i.e. extra-virgin olive oil.

3 main oil/fat types

1. **Saturated** – solid at room temperature  
   e.g. animal fats, dairy, butter  
2. **Monounsaturated** – liquid at room temperature  
   e.g. olive oil  
3. **Polyunsaturated** – liquid at room temperature  
   e.g. flax, rapeseed, sunflower or fish oil

Animal & Saturated fats

Saturated fats are solid at room temperature & in many cases arise from animal sources. They are also found in plants; popular examples are palm & coconut oil/fat. Dairy is a common source of saturated fat, hence why low-fat products have become popular. It is actually hard to completely cut saturated fats from your diet & a small percentage will no doubt have some merit. The problem is so many modern foods contain a large proportion of calories as saturated fat; coupled with an increasingly sedentary lifestyle, this contributes to raised cholesterol & heart disease.

Butter

Butter is a saturated fat that undoubtedly adds taste to a meal, & unlike margarine is a natural product. Eaten occasionally, in combination with an active lifestyle, the negative effects of butter may be minimal. Unfortunately, most of us don’t lead physically demanding lifestyles & eat saturated fats to excess, which can then trigger changes within blood vessels that contribute to damage of the vessel lining (endothelium). Over time this damage (inflammation) leads to fatty deposits in the endothelium which can cause both hardening (arteriosclerosis) & thickening (atherosclerosis) of the vessels, leading to heart disease.

Olive oil

Olive oil is a key ingredient of Mediterranean cuisine. The important thing to remember is the type of oil you purchase or eat. Extra-virgin olive oil is produced from the first cold-pressing of olives, or rather, by mechanical means; unlike oil obtained at later stages produced via chemical extraction, which lacks the anti-inflammatory nutrients (polyphenols) contained in the darker green 1st pressing of the olive fruit. Virgin olive oil generally contains lower amounts of anti-inflammatory polyphenols, & oils labelled olive oil, refined, light, or pomace should be avoided as they offer little health benefit.  

Olive oil has been shown to reduce the level of LDL cholesterol (unhealthy form), when used to replace saturated fat & spreads. Care should be taken when cooking with olive oil as high heat will damage its health properties. Ideally you should cook over a low heat & use sparing amounts. It is better to add fresh extra-virgin olive oil to your meal when serving if you require more.

Olive oil spreads

The marketing surrounding olive oil spreads is important. They are now very popular, yet the packaging image is often misleading, for instance:
The olive oil used in the product is typically low-grade & the packaging will contain terms such as ‘delicate olive oil taste’ or ‘light tasting’.

- Only a small proportion of the spread is made from olive oil
- Even the best (so-called) extra-virgin olive oil spreads still only contain a minimum of 10% saturated fat & a relatively low total percentage of olive oil.

**Essential fats: Omega-3 & 6 oils**

Essential fats are the type your body cannot make itself & must be obtained from your diet. No doubt you have heard of the Omega-oils at some point. Although they are essential, our modern diet has a ratio containing much higher levels of Omega-6. The problem is Omega-6 oils are used by the body to produce substances that can lead to inflammation. This is not a bad thing when inflammation is required, but excessive amounts of these substances in our blood contribute to the development of chronic inflammation. After transplantation, the ideal situation for your body is to have an environment that is more anti-inflammatory, thereby reducing the incidence of organ rejection. Omega-3 oils on the other hand, like those found in fish oil, tend to produce substances that reduce inflammation.

**Fish oils**

There is much interest surrounding the health properties of fish oil. In general these benefits are attributable to Omega-3 fats found in oily fish varieties & have been shown in numerous research studies to lower inflammation. Research also indicates fish oils may potentially reduce the risk of certain types of cancer & improve brain function - this research is ongoing.

**Vegetable Oils: Flax, rapeseed oil, sunflower oil**

*Flaxseed oil* has a lovely nutty taste, but must not be used for cooking as it is unstable at increased temperature. Rather, flaxseed oil is best drizzled fresh over salads, vegetables or a baked potato for example.

*Rapeseed oil* on the other hand (Canola is its other name) has recently become popular with chefs as it has a high cooking temperature, making it useful for frying/stir-frying etc. It must be noted though that the quality of the oil is important. Ideally you want a cold (first) pressed variety, which can sometimes be slightly pricey. Non cold-pressed varieties are chemically refined & offer little benefit - they are best avoided.

*Sunflower oil* is well known to many of us & commonly used for frying all manner of foods. Although good quality sunflower oil is thought to be better for frying (less prone to breaking down at high temperatures), ideally it is better to minimise cooking with frying methods as the process leads to damaged food nutrients & ultimately the development of a range of health disorders. A useful image may be to think of a chip-pan fryer; how difficult is it to wash off the cooked oil? Imagine the effect of that oil running round your blood vessels for many years.

**Margarine**

Margarines are termed trans-fats (transformed fats). Trans-fats are artificial & not found in nature; they are produced through chemical means as a way of making cheap spreads that serve as butter substitutes. Margarines sometimes contain a blend of butter & as such are marketed in a manner to pass them off as butter, such as adding a yellow colour to make them appear buttery. Regular intake is linked to increased blood levels of unhealthy LDL cholesterol & heart disease – avoid whenever possible.

**Oil stability**

It is important to store oils in a cool place out of direct sunlight as heat & direct light lead to oil breakdown. Any benefit that an oil/fat may offer is soon reversed when it becomes rancid. Cooking methods also require careful consideration. As previously mentioned, olive oil is not good for high temperature cooking as high heat damages its nutrients; others such as rapeseed are more stable at higher temperature.
Aim to use a tiny amount of oil during cooking (much easier with non-stick pans), then add fresh uncooked oil (ideally extra virgin olive or flaxseed oil) to the dish when ready to serve.
Breakfast Ideas

Porridge
¾ cup of rolled oats or oatmeal per person

For those not already eating porridge, this is a versatile dish with plenty of benefits. Not only will porridge satisfy the feeling of hunger, it has also been shown to help lower cholesterol as part of a cholesterol reduction plan.

A handy tip when preparing is to pre-soak your oats in the evening. This reduces the cooking time in the morning to minutes, whilst improving digestion & nutrient absorption. Add rolled oats or oatmeal to a pan/pot, barely cover with water & place on lid; leave to soak overnight. When ready to cook, add milk (various choices below) & place over low-medium heat, keep stirring to prevent sticking/burning. Try adding spices as below or chopped hazel or Brazil nuts as it thickens to your desired consistency.

Variations:
- Adding a small amount of dried fruit with the oats in the evening will sweeten the porridge & rehydrate the fruit. A few chopped dried dates also give porridge a lovely sweet taste
- Stewed fruit can be added instead of dried fruit
- In the summer try adding 2-3 tablespoons of mixed fresh or frozen berries (defrosted)
- Warming spices such as powdered cinnamon or nutmeg are great in the winter & help improve digestion
- Add 1 tsp of dark cocoa powder when soaking (or in the morning) for a healthy chocolate taste.
- You could top with a dollop of Greek-style yogurt
- For the adventurous, porridge can be made with other flaked grains such as spelt, rye, wheat, barley or quinoa or try a 50/50 mix of oats & other flaked grain

Muesli
Muesli can make a simple breakfast to keep you going until lunchtime. You can purchase a pre-made (non-sweetened) variety or even better have a go at making your own - then you decide what to add.

Ideally, you want to make muesli with fresh fruit & berries, but either way, the basic recipe for making your own is:
- Mix 1 cup of 3 different dry rolled grains e.g. whole-grain rolled oats, rye flakes, wheat or spelt flakes
- 1 cup of dried fruit, ½ a cup of nuts &/or seeds & 2 tablespoons of cinnamon (mix thoroughly). This will make roughly 4 (¾ cup) servings - or mix a larger batch & store in an air-tight container.

There are 2 main ways to prepare your muesli
1. If you like it crunchy, just pour on your milk of choice (low-fat dairy, soy, almond, hazelnut or rice milk) or try Greek yogurt
2. If you like your muesli softer, soak it the night before to make it easier to eat & digest. Or pour on milk 10-15 minutes before eating whilst making a cup of tea; it will allow the grains to soften slightly.

Variations:
- Don’t be afraid to try new types of nuts, seeds (sunflower, pumpkin or flax) & seasonal fruits
- Drizzle with a little honey if a sweeter taste is desired
Instead of cinnamon, you could substitute with cocoa powder for a chocolaty taste & add some banana slices on top.

**Smoothies**
Do you have a blender in your kitchen? If so, what could be quicker than a smoothie in the morning! Each of the following smoothies are packed with healthy nutrients to help kick-start your day & will serve 2 people.

Just halve the recipe if required for 1 person.

**Super-Berry Smoothie** (serves 2)
200grms or 1-1/3 cup of strawberries, raspberries, black or blueberries (fresh or frozen)
1 tablespoon of ground almonds
1 ripe banana
200grms or 1 cup of Greek-style yogurt
200ml or 2/3 cup of skimmed, soy, hazelnut, almond or rice milk
Chopped almonds or other nuts for topping (optional)

Place all the ingredients into your blender & whizz until smooth. Pour into glasses & add chopped almonds if desired.

*Additions:*
- Try adding 1 tablespoon of flaxseeds (linseed) to your smoothie. Flaxseeds contain beneficial soluble fibre & omega-3 fats which are essential for health. Most health food shops sell flaxseeds, but they are much cheaper when purchased whole (ground are more expensive)
- If you want you can add in a small handful of oat or muesli flakes to thicken & satisfy your hunger

**Mango-Banana Smoothie** (serves 2)
De-skin half a medium/large mango
1 banana
Whole or chopped nuts of choice (almonds, hazelnuts, walnuts, cashews or pecans)
1 cup of Greek-style yogurt
200ml or 2/3 cup of skimmed, soy, hazelnut, almond or rice milk
Chopped nuts as above for topping (optional)

Place all the ingredients into your blender & whizz until smooth. Pour into glasses & add chopped almonds or other nuts if desired.

**Guilt-free Chocolate Smoothie** (serves 2)
2 bananas
1 cup of skimmed, soy, hazelnut, almond or rice milk
2 heaped tablespoons of dark Chocolate/Cocoa Powder
1 cup of Greek-style yogurt

Place all the ingredients into your blender & whizz until smooth. Pour into glasses & add chopped almonds if desired.

**Eggs & whole-grain toast**
Eggs are a great source of protein, rich in vitamins & minerals, yet low in calories! They make a great breakfast & the healthiest cooking options are boiling, poaching or scrambling; you can change the scrambled egg recipe below to boiled or poached eggs if desired.

*Scrambled egg with roasted tomatoes:*
Aim to get some colour on your plate! Try serving scrambled eggs with roasted cherry tomatoes on whole-wheat muffins or toast.

**Pre-heat your oven before cooking your eggs**

Allow a handful of cherry tomatoes per person.
Place tomatoes onto a heated tray, drizzle in olive oil & add a pinch of black pepper.
Place tray in top of oven & cook until tomatoes start to burst.
Garnish with fresh torn basil leaves if available

*TIP when scrambling eggs:*

- Use 1 tablespoon of virgin olive oil instead of butter/margarine. Turn the pan down to a low heat & slowly cook the eggs taking care to stir constantly, by reducing the heat you will produce softer eggs, rather than dry scrambled pieces. You can also add a little skimmed milk in early as you cook - if preferred.

**Omelette**

Omelettes are quick to make & provide a filling breakfast that will see you through until lunchtime.

Preheat your pan on a low-medium heat.
Add a teaspoon of virgin olive oil to reduce sticking
Whisk 2 eggs together (you could add 1-2 tablespoons of skimmed/alternative milk & a pinch of black pepper)
Pour mixture into the pan then sprinkle in chopped vegetables such as sweet/bell peppers or diced tomatoes & wait for the eggs to firm.
If the pan is small and the omelette deep, cover with a large lid; this will help the eggs cook more evenly before flipping over.

*Additions:*

- Tomatoes & peppers go well with omelettes, but try adding fine sliced sugar snap peas, mange-tout or green beans
- Other options to try are steamed spinach leaves, cooked mushrooms or fine sliced spring onions
- Try serving with rocket or watercress leaves

**Healthy Oat Pancakes**

Shop-bought Scotch pancakes contain all manner of unhealthy ingredients, so why not make your own! They are quick & cheap to make & will set you up for the day.

*Ingredients:*

1 & ½ cups of fine rolled oats
2 cups of skimmed, soy, hazelnut, almond or rice milk
1 cup whole-wheat flour
½ tsp of cinnamon powder (optional)
1 tsp. vanilla extract (optional)
2 whole eggs (beaten)

*Preparation:*

Mix oats, whole-wheat flour & milk in a large bowl & let stand for 5 minutes (better still, the night before to really soften the ingredients)
Add eggs & cinnamon (optional)
Mix thoroughly to a smooth consistency
Heat your frying pan on a medium heat & add a drizzle of virgin olive oil (take care not to burn the oil at this stage)
Pour mixture into pan to form mini pancakes & leave to cook. Once they are firming on top, flip over. You could serve with sliced fresh fruit, berries or banana slices & a dollop of un-sweetened Greek-style yogurt

**Lunchtime Ideas**

Individual tastes may vary, but there are a stack of ideas for lunchtime meals, ranging from last minute quick meals made from the previous day’s leftovers, to quick tasty soups. Remember to ask yourself, and this applies to all your meals, are there ingredients in there that not only taste good, but contribute to improving my health? For many people, sandwiches, wraps & soups constitute a midday meal, below are a few ideas to get you started. In many cases, you will be simply tweaking your regular meals to improve them, such as going wholegrain & adding colour with fresh veggies.

**Whole-wheat or rye sandwich**

Keep it simple! A nice wholegrain bread variety with a salad, roasted vegetable, fish or chicken filling takes minutes to prepare.

**Ingredients**

Salad filling can be anything you enjoy, but remember to try new things or prepare ingredients in a different way. Radishes can be sliced thinly to add crunch, carrots can be quickly cut into ribbons with a modern potato peeler, or grated. Salad greens are as simple as rinsing under cold water & shaking dry.

1. Salad; try to mix it up & vary your choices
2. Roasted/grilled vegetables (try preparing more at a previous meal so you have surplus for the following days lunch)
3. Fresh or oily tinned fish (in brine) such as sardines & mackerel
4. Chicken strips can be quickly grilled or cooked in a non-stick frying pan
5. Boiled and mashed egg
6. Drizzle with extra-virgin olive oil
7. Fillings; try hummus or mashed avocado, low-salt peanut butter or tahini instead of butter & spreads
8. Look out for fresh sprouted seeds & legumes, which are not only healthy, but taste delicious

**Whole-wheat tortilla wrap or pitta**

A tasty wrap makes a great lunch & can be prepared similar to the sandwich. Toasted whole-wheat pittas with a roasted veg filling or boiled egg salad are quick to make & filling. Prepare your wrap with similar ideas to the sandwich.

**Green bean, olive & roasted pepper salad**

A Mediterranean-style salad that tastes great in wholemeal pitta-pockets.

**Ingredients**

*Serves 2*

2 medium free-range eggs
200g of trimmed French beans
1 tablespoon of red wine vinegar
1 tablespoon of extra-virgin olive oil
1 small red onion – finely chopped
2 roasted red peppers – finely chopped
A handful of black olives – stoned & halved
Black pepper & sea salt to taste

Add eggs to boiling water for 6-8 minutes. Remove from boil & place in cold water. Peel & slice.
Steam green beans until al dente & then quickly run under cold water to stop the cooking process & retain nutrients – dry on kitchen paper & place in a bowl.
Whisk the vinegar into the oil & season, then add to the beans & toss through. Then stir in the onion, olives, red pepper & egg.

**Tomato & red onion salad**
Easy to make & full of flavour

**Ingredients**
_Serves 2_
1 large tomato or a small handful of cherry tomatoes – diced
½ red onion – sliced fine
1 tablespoon of extra-virgin olive oil
Juice of ½ a lemon
1 tablespoon of balsamic vinegar
Fresh ground black pepper
Small handful of fresh torn basil leaves

Mix all ingredients together in a bowl & serve.

**Rocket & watercress salad**
Both of these salad leaves are rich in vitamins & minerals & provide a delicious peppery taste in salads

**Ingredients**
_Serves 2_
2 large handfuls of rocket
1 large handful of torn/snipped watercress
1 gem lettuce – torn
1 tablespoon of cress (if available)
1 tablespoon of extra-virgin olive oil
1 teaspoon of balsamic vinegar (optional)

Combine all ingredients & mix together – adding the dressing

**Warm puy lentil, carrot & hazelnut salad**
A warm salad that can be enjoyed throughout the year, but it’s especially ideal in winter.
Experiment by adding fresh herbs such as basil or chives, crumbled cheese (feta), diced tart apple or different nuts such as pecans or walnuts.

**Ingredients**
_Serves 4_
250g of dried green lentils soaked for 8 hours (if rushed use tinned)
50g of whole hazelnuts
3 tablespoons of balsamic vinegar
3 tablespoons of extra-virgin olive oil
1 large garlic clove – or 2 small
½ a red onion – sliced fine
3 carrots – peeled & grated
3 tablespoons of chopped parsley – coarsely chopped
Ground black pepper & sea salt

Empty soaked and drained lentils into a pan, cover with water & bring to the boil. Once boiling, reduce heat then cover & simmer until lentils are soft but *al dente* (roughly 15 minutes). Place hazelnuts into a clean kitchen towel & crush coarsely with a rolling pin. Next, mix vinegar, oils & garlic in a mixing bowl. Add onion & carrot to the dressing. Once lentils are cooked, strain into a sieve & add to the salad bowl. Mix everything together & allow vegetables to warm through. Garnish with chopped parsley & nuts.

**Soups**

Soups offer many advantages; they are easy to make, can be batch prepared & are easy to digest & absorb. Ideally, you want to make soups in larger batches so you can store in the fridge for the following day, or freeze in containers for those days when you want a quick meal or are feeling under the weather. If you have not prepared a soup before, don’t worry it’s not difficult, here a few recipes below & you will soon be making your own up. As an additional note, try adding a drizzle of extra-virgin olive oil to your soup when serving to ensure you are consuming an adequate daily amount.

**Vegetable soup for beginners**

This recipe contains much of the basic information to make a soup & it’s simple to prepare. You can add all manner of vegetables (e.g. bell peppers, celery) & if you want to add in leftover cooked chicken it will provide extra protein. You could try adding a few chunks of marinated tofu to give it a meat-free edge?

**Ingredients**

1 tablespoon extra-virgin olive oil (can add extra when cooked as a drizzle)
1 large red or yellow onion finely chopped
2 cloves crushed garlic
2 large carrots – peeled & finely cubed
2 leeks – wash & slice finely (including the green sections)
100g of pre-soaked haricot beans (if tinned – drain & rinse)
1 small head of broccoli – coarsely chopped
1 litre (2 pints) vegetable or chicken stock
1 pinch of thyme
1 bay leaf
2 tablespoons of chopped parsley
Drizzle of lemon juice (optional)
Freshly ground black pepper & sea salt to taste

In a large, heavy cooking pot, warm 1 tablespoon of extra-virgin olive oil on a medium heat & add the chopped onions, 1 clove of garlic, carrots & leeks. Cook for 5 minutes (stirring regularly); add in the haricot beans, hot stock, bay leaf & thyme. Simmer covered on a low heat for 10 minutes, or until carrots are soft.
Add the chopped broccoli & cook for a further 5 minutes. Using a garlic press, squeeze in the second clove of garlic (or add finely chopped). Remove bay leaf, transfer the soup to a blender (or immerse a stick blender) & puree until your desired consistency is reached. Serve & sprinkle the chopped parsley, season to taste with pepper, salt & lemon juice if desired. To increase your intake of extra-virgin olive oil – drizzle on top.

**Carrot & Coconut Soup**
Ginger, orange juice and coconut milk transform ordinary carrots into a sensational soup that’s quick to prepare.

*Serves 4*
1 kg carrots
2 yellow onions
2 cloves of garlic
1 tablespoon ginger - peeled and chopped
2 teaspoons of extra-virgin olive oil
1 litre of water
200 ml coconut milk
1 teaspoon sea salt
½ teaspoon white pepper
Juice from one Orange
4 tablespoons low-fat yogurt (or Greek low-fat)

Peel and chop the carrots, onions, garlic and ginger. Heat oil in a soup pot & cook vegetables until tender, about 10 minutes. Add water, coconut milk, sea salt & white pepper & simmer for 20 minutes. Transfer mixture to a blender in batches & puree until smooth, then add fresh squeezed orange juice. Serve topped with one tablespoon of low-fat yogurt per person.

**Broccoli soup**
One of the quickest & tastiest soups you can prepare. You need to keep an eye on this dish though to ensure you do not overcook the broccoli & destroy both taste & beneficial nutrients.

**Ingredients**
2 tablespoons of extra-virgin olive oil
1 small red or yellow onion – finely sliced
1 celery rib – finely chopped
1 clove of garlic – finely chopped
1 large head of broccoli – coarsely chopped (including the stalk)
1 bay leaf
1 pinch of thyme
750ml (1 & ½ pints) of vegetable or chicken stock
Freshly ground black pepper & sea salt
Lemon juice (optional)
Red pepper flakes or paprika powder

In a heavy cooking pot on a medium heat, warm the olive oil & sweat the onion, celery & garlic for 4-5 minutes until the onion is translucent. Add broccoli, herbs & stock, bring to the boil, reduce heat, cover & cook for 6-7 minutes.
When the broccoli is soft (but *al dente*) remove from heat & take out bay leaf & transfer vegetables & stock to a blender in batches & puree until smooth. Add a small quantity of hot water if necessary. Season to taste with pepper, sea salt, paprika &/or red pepper flakes. Perhaps add a dollop of Greek/low-fat yogurt.

**Spring Vegetable Frittata**

Load this frittata up with a variety of fresh vegetables for a dish that goes great at lunch, dinner or taken along on a picnic. Serve it up with a refreshing salad to complete the meal.

**Ingredients**

*Serves 8* (adjust recipe, or refrigerate & eat the follow day for lunch)

- 1 courgette - chopped
- 125 g green Peas
- 1 pint cherry tomatoes - cut in half
- 1 head broccoli - cut into florets
- 1 bunch asparagus - cut into 2 cm long pieces
- 1 carrot, peeled & thinly sliced
- 1 Leek - chopped and washed
- 500 ml low-fat milk or soymilk (unsweetened)
- 8 Eggs
- 2 teaspoons of sea salt
- 1 teaspoon black Pepper

Preheat oven to 175 degrees. Lightly oil a non-stick frying pan or baking pan and add courgette, green peas, tomatoes, broccoli, asparagus, carrots & leeks & stir to mix. In a mixing bowl whisk together eggs, soymilk, salt and pepper & pour over vegetables. Bake until the centre is set - roughly 60-70 minutes.

**Evening Meal Planner**

There’s literally no shortage of recipes to choose from for an evening meal. Most recipe books & webpages contain Mediterranean-themed meals that if not already healthy, can be easily adjusted. Perhaps one of the most important points to bear in mind is that you are not being asked to stick solely with a Mediterranean diet. You can prepare all manner of meals from Indian curries to Thai dishes; you just need to remember to think about the ingredients you are including & aim to preserve as many nutrients as possible – using colour as a simple indicator. It may be easier to start by modifying some of the meals you currently eat & transform them into healthier options. Here are a few ideas below to get you started.

**20 minute seafood pasta**

Simple tasty meal that cooks in no time!

**Ingredients**

- 1 tablespoon of extra-virgin olive oil
- 1 red or yellow onion – sliced fine
- 1-3 cloves of garlic – depending on your taste
- 400g tin of chopped tomatoes
- 1 litre of vegetable or chicken stock
300g of whole-wheat pasta – roughly broken
250g of mixed seafood – e.g. prawns, fish chunks – your choice
1 teaspoon of paprika
Black pepper & sea salt to taste
Chopped parsley

Add onion, garlic & olive oil to a non-stick pan. Cover & gently heat until soft & translucent. Add stock, paprika & pepper & then bring to boil. Reduce heat to a simmer then stir in pasta pieces & cook for 5 minutes (stir occasionally to stop pasta sticking). Add seafood pieces & cook until pasta & seafood are cooked. Season to taste & add chopped parsley & serve with a handful of rocket leaves.

**Rice & vegetable stuffed aubergine**
Stuffing vegetables is a great way to double your serving of vegetables. Not only do you get the benefits of the vegetable that is stuffed, but also all the healthy whole-grains & vegetables it’s packed with. Bell peppers, courgette, tomatoes & onions are other great fillings.

**Ingredients**
*Serves 4*
2 small aubergines cut in half & inside removed
1 teaspoon extra-virgin olive oil
1 red onion - sliced
1 red bell pepper - sliced
2 cloves garlic - chopped
100g Mushrooms - sliced
100g green peas
100g tomatoes - chopped
300g cooked brown rice
½ teaspoon sea salt
¼ teaspoon black pepper
1 tablespoon parsley - chopped
4 tablespoons Parmesan cheese – grated (optional)

Preheat oven to 200 degrees. Heat the oil in a skillet & sauté the onion, bell pepper, garlic & mushrooms until tender & lightly browned; roughly 8-10 minutes. Toss cooked vegetables with peas, tomatoes, brown rice, salt, pepper & parsley & divide mixture amongst the four aubergine halves. Bake until aubergine is softened, around 15 minutes. Top each with one tablespoon of grated parmesan & continue baking until cheese is melted & golden brown, about 2-3 more minutes.

**Sweet-potato omelette**
Sweet potatoes are richer in nutrients & have a lower glycemic index than white potatoes, which is more beneficial for blood sugar levels. Feel free to add other ingredients such as diced red or green bell peppers, courgette, peas, olives or finely sliced chillies. For extra seasoning & colour, try adding paprika & turmeric.

**Ingredients**
2 tablespoons of extra-virgin olive oil
1 large red or yellow onion - coarsely chopped
4-5 cloves of garlic – finely chopped

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2 medium sized sweet potatoes – peeled & cut into half inch cubes
4 eggs
½ teaspoon of finely chopped rosemary
Black pepper & sea salt to taste

Place sweet potato pieces into a steamer & leave to steam whilst preparing onions. Add onion, garlic & 1 tablespoon of olive oil to a non-stick pan (cover & sauté until soft). Meanwhile, break eggs into a mixing bowl & beat with a fork (or stick blender for ease). Season with pepper & salt, add rosemary. When the potatoes are *al dente* & onions/garlic translucent, remove from heat, cool slightly then tip into the egg mixture & gently combine. Add 1 tablespoon of olive oil to a non-stick frying pan & heat. When hot, pour in contents of the bowl & spread evenly, turning the heat down to the lowest setting. Cook gently for 8-10 minutes until the underside of the omelette is golden brown – the top may still be wobbly. If so, slide the omelette onto a large plate & flip over so that the cooked side is now facing upwards in the pan. Return to heat & cook for a further 2 minutes. You could also do the second stage under a grill if easier – though mind the handle. Serve with a nice mixed salad of your choice.

**Mediterranean fish parcels**
Steaming in foil is a great way to cook fish & vegetables together, leaving them deliciously moist. Best of all there is little to wash up when you have finished eating. Add some cooked whole grains or legumes & a salad to round out the meal. Feel free to vary the recipe with a range of colourful vegetables.

*Serves 4*
4 100 g fish fillets – oily or white fish
1 fennel bulb - sliced
1 courgette - sliced
1 red or yellow onion - sliced
1 red bell pepper - sliced
100 g mushrooms - sliced
200 g cherry tomatoes cut in half
1 lemon - thinly sliced
2 tablespoons chopped dill
½ teaspoon sea salt (optional)
Fresh ground black pepper
4 large sheets of Aluminium foil

Preheat oven to 200 degrees. Lay out foil & fold up the edges slightly to keep everything in. Divide the vegetables among the four pieces of foil & layer in the middle of the foil & season with salt & pepper. Place a fish fillet on each packet, season with salt & pepper, place on lemon slices & chopped dill. Add 1 tablespoon of water to each piece of foil & fold edges together to seal. Place in oven & bake until fish is cooked, about 12-15 minutes for thin pieces of fish & 18-22 minutes for thicker pieces.

**Oily fish dishes**
Oily fish are a regular addition to many Mediterranean tables. Fish such as mackerel, sardines, herring & pilchards are readily available, good value & most importantly, rich in essential omega-3 fats. They can be cooked in all manner of ways, but grilling, dry pan frying (non-stick) & oven roasting are some of the tastiest & quickest methods. What could be
easier than grilled mackerel, garlicky new potatoes or butterbeans & a fresh salad drizzled in extra-virgin olive oil & cracked black pepper.

There are times when you may fancy a light meal. Smoked herrings cook in 2 minutes in boiling water & can be placed on fresh toasted wholegrain bread with a salad of your choice, or fresh cherry tomatoes.

**Veggie chilli**
A classic dish packed full of healthy ingredients with a healthy twist that will satisfy both vegetarian & meat eaters alike. Serve with a portion of cooked whole-grains or even roasted sweet potato wedges.

**Ingredients**
**Serves 4**
2 red or yellow onions - finely chopped
2 garlic cloves – chopped fine or pressed
1 tablespoon of extra-virgin olive oil
½ teaspoon ground cumin
1 teaspoon chilli powder
½ teaspoon smoked paprika
1 handful coriander - chopped
1 teaspoon oregano or marjoram (if available)
1 tin of chopped tomatoes
1 teaspoon tomato purée
½ cup vegetable stock
1 tin of kidney beans, drained & rinsed (or try mixing different beans)
1 large red pepper - diced into strips
Handful of mushrooms - sliced
1 cube dark chocolate (optional)
Salad leaves to serve e.g. rocket or watercress

Sauté the onions in a sealed pan until tender, then add in garlic & continue cooking until translucent – stir occasionally to avoid burning. Add the olive oil, cumin, chilli, paprika, coriander & cook for 1 minute to release the flavours. Add tomato puree & cook for a further minute. Add the tinned tomatoes, red pepper, herbs & vegetable stock. Season with salt & pepper, then cook for 10 minutes - stirring regularly. Add in kidney beans & mushrooms, simmering for a further 10 minutes. Finally, add the coriander & chocolate & stir until melted. Serve with brown rice or wholegrain of choice.

**Fragrant chicken curry**
Lean chicken can be transformed into a low-fat healthy curry. Spices & herbs provide flavour, without excess calories. That’s correct it’s not Mediterranean, but it doesn’t matter one bit, it’s the ingredients & preparation that counts!

**Ingredients**
**Serves 4**
1-2 red or yellow onions – quartered
2 garlic cloves – large
1 large thumb-sized piece of ginger – peeled & rough chopped
2 tablespoons curry powder (medium strength)
½ teaspoon turmeric
1 teaspoons paprika
½ teaspoon of black pepper
1 fresh red chilli – de-seeded & rough chopped
1 pack or bunch of fresh coriander
500ml chicken or vegetable stock
3 chicken breasts or 4 chicken legs or thighs (de-boned & skinned)
1 tin (400) of chickpeas – drained & rinsed

Add onions, garlic, ginger, curry powder, ground spices, chillies & half the coriander into a food processor. Add a touch of sea salt & blend to a puree. This may need to be done in 2 batches. Pour contents into a large pan & cook over a low heat for 10 minutes, stirring regularly. Make up 500ml of stock, add to pan & return to heat. Add chicken pieces, then lower heat to a simmer & cook for 20 minutes or until chicken is tender. You may need to add a little water if it’s over-thickening. Chop the remaining coriander & then stir in at the last minute before serving. Serve with a wholegrain of choice & a spoonful of low-fat yogurt if desired.

**Side dishes**

**Butternut mash**
As a side dish the squash family make a great accompaniment to a range of meals. This mash is both rich in nutrients & offers a lower glycemic index than white potatoes, with the benefit of being quick to prepare. For a more exotic flavour, try adding 1 teaspoon of grated fresh ginger.

**Ingredients**
- 1 butternut squash (or any other variety)
- 2 cloves garlic – finely chopped
- 2 tablespoons extra-virgin olive oil
- 2 tablespoons parsley - chopped
- Freshly ground black pepper & sea salt

Scrub the squash on the outside under running water using a soft brush. Remove the stem from the end & discard & then cut the squash in half. Remove seeds. Chop into 1 inch cubes & place in a steamer & cook until soft (10-15 minutes). With a hand-held stick blender (or processor), mash the squash & add in garlic & olive oil until you have a creamy puree. Garnish with chopped parsley if available

**Cauliflower mash**
A great twist on a family favourite to reduce calories without the expense of taste. Adding anti-inflammatory spices such as turmeric provides a spicy dash of colour to accompany a range of dishes.

**Ingredients**
- Serves 4
- 3 tablespoons of extra-virgin olive oil
- 1 red & yellow onion – chopped fine
- 2 cloves of garlic
2 leeks – sliced
1 teaspoon of turmeric
1 cauliflower – coarsely chopped
100ml of vegetable stock (or water)
Squeeze of lemon juice (optional)
2 tablespoons of chopped parsley
Fresh ground black pepper & sea salt

In a heavy bottomed pan, heat 1 tablespoon of olive oil & add onion, garlic & leeks, salt lightly & cook for 5 minutes. Add turmeric & pepper & cook for a further minute, stirring. Add cauliflower & stir well to coat the cauliflower evenly with the golden colour of turmeric. Add stock or water, cover & cook for 10-12 minutes until soft. Blend with a stick blender (or processor) until mashed. Season with pepper, sea salt (lemon juice is desired) & remaining olive oil, mix through & garnish with chopped parsley.

**Garlicky broccoli**
Simple & quick, steamed crunchy broccoli tossed with garlic will accompany many dishes & tastes fabulous. Try garnishing with pine nuts or toasted sesame & sunflower seeds.

**Ingredients**
1 head of broccoli – chopped into small florets
2 tablespoons of extra-virgin olive oil
2-3 cloves of garlic – fresh pressed
¼ teaspoon of chilli flakes (optional)

Place broccoli in a steamer or pan of boiling water. Cook for 3 minutes until tender but retaining that vibrant green colour. Remove from heat, drain & place in bowl. Drizzle with olive oil & season with black pepper, a touch of sea salt & chilli flakes.

**Ratatouille**
Simple & tasty, this French dish includes a range of vegetables & its hearty flavour complements salmon & chicken, or as a filling for vegetable lasagne.

**Ingredients**
Serves 4
1 courgette
1 aubergine
1 red or yellow onion
1 red bell pepper
2 cloves of garlic – finely chopped
1 tablespoon of extra-virgin olive oil
½ teaspoon dried thyme
½ teaspoon dried oregano
1 can (400g) chopped tomatoes
1 teaspoon of sea salt
½ teaspoon of freshly ground black pepper

Cut courgette, eggplant, onion & bell pepper into approximately 2 cm cubes. In a heavy bottomed pot, heat oil over medium high heat. Add courgette, aubergine, onion, bell pepper & garlic & cook until lightly browned, about 10-12 minutes. Add thyme & oregano & cook
for one minute before adding remaining ingredients. Turn heat to low & cook until liquid is cooked down to a stew-like consistency, about 15 minutes.

Sofrito Sauce

“Sofrito” is a tomato-based sauce commonly eaten in the Mediterranean (especially Spain) & serves as a base for many dishes. As with all traditional recipes, methods vary depending on family secrets, but the basic ingredients are pretty much the same. It is simple to make & tastes delicious, but just as important, it contains ingredients that are beneficial to our health. Tomatoes, onions, garlic & extra-virgin olive oil provide the basic ingredients. Try out the recipe below & adjust the ratios to suit your taste.

Similar to a curry, this sauce will mellow & taste better the following day. This recipe will provide more than enough for 2 people, so left overs could be eaten for lunch the following day. Failing this, make a larger batch and store in a sealed container in the refrigerator - it will keep well for a few days.

Ingredients:
- 1 tin or carton of tomatoes
- 2 red or yellow onions (sliced fine)
- 2-5 cloves of finely sliced fresh garlic
- 1tsp of sweet paprika
- Black pepper
- Long, green & mild sweet pepper (optional)
- Sea salt to taste (optional)
- Extra-virgin olive oil

Preparation:
- With a sharp knife, finely chop your onions & garlic & simmer in a non-stick pan with closed lid. Your basically sautéing until transparent on a very low heat to avoid burning. Keep an eye on this stage & stir occasionally – remember to replace the lid. It should take 5 minutes.
- When soft & reduced, add in the rest of the ingredients, chopped tomatoes & stir well. Simmer for 10-15 minutes – stirring occasionally.
- Add olive oil at the end of the cooking process – that way you will get all the taste & minimise the damage to those healthy olive antioxidants.
Salad Dressings & Sauces

How do you add taste to a dressing or sauce without the calories of butter, mayonnaise or cream? Don’t panic, we have prepared a few delicious recipes for you below. They are quick to prepare & a 300ml bottle will dress plenty of salads & ensure you incorporate regular amounts of extra-virgin olive oil into your diet. The other way to use them is to dress steamed vegetables such as green beans, mange tout, broccoli & asparagus to add a touch of zing! Sauces such as Sofrito will again transform many dishes, & hummus makes a tasty snack with oat cakes or carrot sticks.

Classis French vinaigrette

Ingredients
300ml of extra-virgin olive oil
100ml of red-wine vinegar
2 teaspoons of honey (optional)
1 tablespoon of whole-grain mustard
Pinch of fresh or dried herbs such as sage, thyme, rosemary, marjoram
Freshly ground black pepper (touch of sea salt is optional)

Combine all ingredients & pulse in a blender or simply add to a clean glass bottle & shake well. Store in the fridge & shake before use

Italian dressing

Ingredients
300ml of extra-virgin olive oil
60ml of lemon juice (or lime)
2 tablespoons of white wine vinegar
2 teaspoons of honey (optional)
1 clove of freshly crushed garlic
Freshly ground black pepper (touch of sea salt is optional)

Combine all ingredients & pulse in a blender or simply add to a clean glass bottle & shake well. Store in the fridge & shake before use. Will keep for 2 weeks

Creamy lemon dill dressing

This simple & satisfying dressing takes minutes to prepare. We substituted the high-fat crème fraîche for a creamy yogurt, which has a rich texture with a fraction of the fat & calories.

Ingredients
100ml of plain low-fat yogurt
2 tablespoons of fresh chopped dill (or try substituting with coriander or parsley)
Zest & juice of a lemon (or lime)
Freshly ground black pepper & sea salt to taste

Stir together & serve

Mango Salsa

Fresh fruit makes a great base for a bright salsa to blend with salads, or to top grilled or steamed vegetables & fish
Ingredients
1 mango – peeled & diced
¼ red bell pepper – finely chopped
¼ green bell pepper – finely chopped
25g or ¼ of a small onion – finely chopped
Juice of half a lime
1 tablespoon of chopped coriander
Freshly ground black pepper & sea salt to taste

Toss all ingredients together & serve

Avocado aioli
Inspired by a southern French garlic mayonnaise & can be used in a similar manner. Creamy & rich, yet guilt-free!

Ingredients
1 ripe avocado
1 tablespoon of lemon juice (or lime)
1 clove of freshly crushed garlic
1 tablespoon of extra-virgin olive oil
Freshly ground black pepper & sea salt to taste

Walnut tarator
Tarator is a garlicky walnut paste from Turkey & Lebanon. It makes a great topping for steamed or grilled vegetables, poultry or fish. Try it as a dip for raw vegetables. Makes 8-10 tablespoons

Ingredients
100g of walnuts (or a 50/50 split with pine nuts)
1 large clove of freshly crushed garlic
4 tablespoons of extra-virgin olive oil
2 tablespoons of lemon juice (or lime)
1 teaspoon of dill or parsley – freshly chopped
Freshly ground black pepper & sea salt to taste
If too thick, add 1-2 tablespoons of water

Add nuts, garlic, olive oil & lemon juice into a small blender & pulse until creamy. Add water to the desired consistency. Stir in dill or parsley & season with pepper & sea salt. Refrigerate in an air tight container – will last for a week.

Hummus
There are 2 options here: If you have time, soak & cook the chickpeas yourself, or else use pre-cooked tinned chickpeas to reduce preparation time. Serve with fresh vegetables for a dipping snack.

Ingredients
1 tin (400g) of chickpeas – drained & rinsed
1-3 cloves of fresh chopped garlic (depending on your taste)
Juice of half a lemon
½ teaspoon of sea salt
¼ teaspoon of fresh ground black pepper
½ teaspoon of cumin powder
2 tablespoons of water

Place all ingredients in a blender & pulse until smooth. If required, adjust consistency with small amounts of water – 1 teaspoon at a time.

**Salsa**
This simple salsa is a great addition to fish or chicken, or tossed with some cooked whole grains to provide a touch of zing. Include the chilli if you like, or omit it for a milder but still delicious salsa.

**Ingredients**
*Serving size 2 tablespoons*
2 tomatoes - finely chopped
¼ red or yellow onion – finely chopped
1 clove of freshly pressed or chopped garlic
1 tablespoon chopped fresh coriander
Juice of half a lime
¼ teaspoon of black pepper
¼ teaspoon of sea salt
½ a jalapeno or serrano chilli, remove seeds & finely slice (optional)

Combine all ingredients in a medium bowl & mix well. Refrigerate until serving.

**Tzatziki**
This creamy & vibrant sauce works well as a thick dressing on a salad, draped over grilled vegetables or to accompany an Indian inspired curry.

**Ingredients**
*Serving size 2 tablespoons*
100ml natural thickened yogurt (or Greek yogurt)
½ cucumber, peeled & grated
1 clove of finely chopped garlic
Juice of ½ a lemon
1 tablespoon of fresh chopped parsley
Fresh ground black pepper & sea salt to taste

In a strainer, squeeze out the excess water from the cucumber. In a small bowl, stir together all ingredients until blended.
Snack Ideas

Hunger can strike at any time; the trick is having healthy snacks at hand to nibble on when you do start to crave food. Skipping meals can increase hunger & this makes it more likely that you will reach for the Hobnobs or a bar of sweet chocolate, rather than a banana, apple or oatcakes & humus etc.

What’s ‘GI’?

‘GI’ is the abbreviation for ‘glycemic index’; it measures the rate at which a carbohydrate food ‘burns’ & releases sugar into your bloodstream. Without getting caught up in technical jargon, here is a quick analogy. If you imagine eating a mars bar or a slice of white toast with jam; these sorts of foods are classed as ‘high GI’ & are used rapidly as energy – or like a grass fire, burn quickly. This causes your blood sugar levels to increase rapidly, which requires lots of insulin to be produced. Over time, high blood sugar/insulin levels contribute to the development of heart disease, diabetes & many other problems.

Lower GI foods on the other hand release their energy (or burn) much slower, which means blood sugar levels do not rise as high/quick as with high GI foods. If you think of a log burning on a fire, it doesn’t burn as quickly as the grass fire, but releases its energy more evenly.

Lower ‘GI’ snacks

- The humble banana
- Fresh fruit – especially apples, pears, & stone fruits (peaches, nectarines, apricots, plums & cherries)
- Dried apricots
- Unsalted nuts/mixed nuts (could add in a small amount of raisins or dried fruit)
- Oatcakes or rye crackers & hummus
- Oatcakes & avocado
- Carrot / celery sticks & hummus, avocado dip or tomato salsa
- Carrot / celery sticks & nut butter (tahini, almond, cashew or peanut)
- If you must reach for a piece of chocolate, choose dark varieties with a minimum of 70% (ideally higher) cocoa content.
- Low-fat Greek yogurt

Hints & tips for reducing the GI of your diet

- Aim to eat at least 5 servings of vegetables & 2 of fruit each day. Vegetables & fruit are essential foods in a low GI eating plan. Outside of breakfast, try to ensure that half of your plate is made up of vegetables – ideally with a range of different colours.
- Changing those sugar-rich breakfast cereals to lower GI options such as muesli, porridge or wholegrain toast will significantly reduce your morning glucose & insulin levels & help set you up for the day.
- Aim to reduce your intake of refined flour products. Supermarket foods such as cakes, biscuits/crackers (not rye), white bread, crumpets, pastries & pies will all affect your GI – even if they state ‘low-fat, low-sugar’.
- Change your bread & start eating wholemeal, yes the ones packed with bits of whole-grains. Examples are stoneground & granary, or soy & linseed. For the more adventurous, try dark rye sourdough or pumpernickel. All these types of bread when prepared have a much lower GI profile than refined varieties. Once you change, you will notice those refined white breads start to taste bland.
- Try to include at least one low-GI carbohydrate with each meal. You will find them in the 4 key food groups:
  1. Fruit & vegetables
  2. Whole-grain bread & cereals
  3. Legumes/pulses (e.g. lentils, chickpeas & lentils)
4. Low-fat dairy foods & soy alternatives

- Embrace the humble legume; many of the world’s longest lived populations eat them regularly. Legumes are really versatile & allow you to make nutritious meals from dried or tinned varieties. Add lentils to soups, kidney beans to chilli con-carne, chickpeas to stir-fries or an assortment of beans to a salad or casserole.
- Potatoes can be high GI foods, especially when mashed – try smaller waxy salad potatoes with a small corn cob, or try a 50/50 split with mash potato & cannellini beans etc.
- Choose starchy foods with a lower GI such as whole-wheat pasta, or grains such as bulgar, quinoa, buckwheat, whole rye, barley or oats. Longer grained rice varieties are generally lower GI. Brown basmati rice has probably the lowest GI, with a high nutritional content (though white basmati is also low GI).
- Don’t forget portion size. Just because a food may have a lower GI, eating more of it will still raise your blood sugar to unhealthy levels. Cooking your grains or pasta al dente (slightly firm) & adding crispy steamed vegetables or salad will transform a meal & positively change the GI of your meal.

**Beverages**

We all have our preferences, yet some drinks are healthier than others. This page aims to offer some simple pointers regarding daily fluid intake & will help complement your healthy eating plan.

**Water**

Some drink plenty, others very little, yet water is essential for life; in fact it makes up 60% of our total body weight. Your daily intake depends on many factors which include activity, health, age & weight. Water carries nutrients into cells (remember blood is liquid) & helps remove toxins & waste products. Dehydration on the other hand is a lack of water - even mild dehydration will reduce energy levels & your ability to focus. Drinking an adequate amount helps kidney function, especially when taking regular prescription medication. Keeping adequately hydrated also aids regular bowel movement, which is a vital daily body function.

**How much water should I drink?**

There are no conclusive daily intake figures for water intake (according to various studies), yet a general daily guide is 9-10 cups for a man & 7-8 for a woman. Bear in mind you will naturally drink less on a cold day, whereas gardening on a hot summers day will soon make you thirsty. All drinks are made up of water, it’s the additives that go in there that can be problematic, such as sugar, sweeteners, colourings & flavours commonly found in cordials & fizzy drinks. If you are not already doing so, try to drink at least a couple of fresh glasses of water each day.

**Tea & Coffee**

Tea & coffee are British staples & many of us will have our favourite. Milk & sugar are commonly added, with semi-skimmed & skimmed milk becoming more popular than full-fat versions. Reducing the fat of whole milk has advantages if you drink many cups each day. Both black tea & coffee beans contain antioxidants, which consumed in moderation seem to offer more benefit than harm. Just take care not to over-do it as both contain the stimulant caffeine.

Green tea has become rather popular - it is actually the same plant as regular (black) tea but its processed in a different way. Both varieties contain beneficial antioxidants (polyphenols), but one of the most researched forms is found abundantly in green tea. White tea is similar to green, but the shoots are picked earlier & not partially fermented; they are said to contain the highest antioxidant levels & less caffeine than black or green tea.
You can buy green & white teas natural or flavoured with citrus or berry fruits, or you could try adding your own twist with a fresh squeeze of lemon, lime or orange.

**Grated Ginger**

For those who enjoy the taste of ginger, a fresh piece makes a great winter-warmer. Ginger has a range of health benefits & research demonstrates it helps protect the heart & blood vessels, whilst its natural anti-inflammatory nutrients have been shown to ease the pain of osteoarthritis. Ginger can also help ease the feeling of nausea; this age old remedy has recently been studied with chemotherapy patients & received positive results.

To make ginger tea, peel a thumb-end sized piece of ginger & grate finely into a cup or small teapot & add hot water. A squeeze of lemon or lime adds a great zingy taste & you could even add a little honey if preferred.

**Sweetened Drinks**

Drinks sweetened with sugar not only contain calories, they also quickly raise blood sugar levels. Reducing (or stopping) added sugar in hot drinks is beneficial & will help remove empty calories. Products such as ‘Stevia’ are an alternative option for those with a sweet tooth. Stevia is a sweet tasting extract derived from the leaves of the South American *Stevia* plant. It has zero calories & is 200 times sweeter than sugar; in the UK it can be bought under the name ‘Truvia’ or ‘Purevia’ & is safe for diabetics. Stevia can also be used as an alternative baking sweetener if required.

**Red Wine**

Over the last two decades wine has become popular in the UK, yet it is the red variety that attracts the attention of researchers. For the purpose of the Mediterranean diet study we are interested in alcohol derived from dark grapes, albeit red wine. Drinking a couple of glasses of red wine per week can be a favourable way of adding antioxidant nutrients to your diet. It is important to note if you do not already drink red wine, do not feel you must start because you are eating a Mediterranean-inspired diet, nor are we advocating excessive drinking; more than a single glass per day may actually reduce the health properties of red wine.

Red wine is made from dark coloured grape varieties, rich in polyphenol antioxidants that are concentrated in the wine making process. Observational studies suggest moderate red wine consumption is associated with reduced cardiovascular disease incidence & attributable to its antioxidant nutrients. Aim to purchase an un-sweetened red wine; the wine does not have to be expensive, there are plenty of well-priced choices in your local supermarket & special offers run all the time. Dry red wine is favoured due to a lower sugar & higher antioxidant content. Semi-dry wine contains more sugar but is still better than a sweetened version, which will also be surprisingly high in calories. The taste of wine will change if sipped with a meal; dryness will be less noticeable if you are used to drinking sweeter wines.
Dining Out: Hints & Tips

Basic tips

- Try splitting a starter, main or dessert with a companion
- To avoid overeating, ask for the lunch portion, even if you're eating dinner
- Substituting an appetizer for a main-meal will reduce portion size or calories
- Salt undoubtedly enhances flavour, but is often excessively used in restaurant meals
- Be aware of ingredients, cooking styles & labels indicating a dish may be high in salt, soy sauce & broth, or contain pickled, cured or smoked ingredients
- Ask that your food be prepared without added salt, MSG or salt-containing ingredients (or with a reduced quantity)
- Ask for lemon, olive oil or fresh herbs for dressings or seasonings
- Always aim to eat slowly & chew your food properly

Starters

- Choose appetizers that feature healthy vegetables, fruits or fish; good choices are mixed salads, raw or steamed fish or shellfish, grilled/baked vegetables or broth-based soups
- To reduce calories; ask that the bread & butter basket not be brought to the table. Alternatively, ask for whole-grain bread, rolls or breadsticks (if available)
- Avoid anything fried. If you’re not sure if a starter is fried - just ask!
- To reduce your portion size, share one starter with 2 or 3 people. Skip the starter altogether if you feel up to it or plan to order a larger main meal

Main course/Entrées

- Again, avoid fried foods
- Share an entrée with a companion or order another starter if you would like to reduce your food portions
- Choose from vegetarian, fish (preferably oily), shellfish or lean poultry options
- If you do order meat, choose lean cuts, eat a portion that's about the size of a deck of cards & ask for any visible fat to be trimmed off
- Ask if they have wholegrain products instead of refined white pasta, rice, bread etc
- If whole-grain options are not available, you can ask for extra vegetables instead, most places will gladly oblige.
- Be aware of sauces used in the dish. Ask questions: Is there cream, butter or oil in the sauce? If the answer is yes, you could choose something else, or ask if it can be made with a healthier ingredient (i.e. extra-virgin olive oil). For example, if you order a pasta dish listing an Alfredo sauce, ask if you can substitute the dish with a marinara (tomato, garlic, olive oil & herb) sauce etc
- Remember many restaurants will alter their dishes according to your dietary needs, so don’t be afraid to ask for specific dietary requirements. If fact most restaurants are now well aware of food allergies etc and & will aim to please
- Try & avoid high-fat sauces made up of butter, cream or cheese (i.e. Alfredo)
- Order vegetables cooked in a healthier way i.e. steamed, baked, roasted, grilled/broiled, poached or stir-fried. Aim for the bulk of your meal to be vegetables
- Substitute high-fat side dishes with healthier options: baked potato, steamed brown rice, side salad, or steamed vegetables (instead of fries/chips), mashed root vegetables or squash, low-fat potato salad or coleslaw
If choosing baked or mashed potatoes; ask for virgin olive oil instead of butter/margarine
If you do order side sauces or salad dressings, dip your fork in the dressing & use the fork to pick up a piece of food. This helps you reduce the amount of sauce or dressing you eat
If the meal is large, or you feel full, you can always ask to take the remainder home. This is good practice for controlling food portions (a key to successful weight loss)
As soon as you feel like you have had enough, call someone over to take your plate away. This will prevent picking on food whilst you socialise at the table

**Desserts**

If you want a dessert; Ideally a healthier option would be mixed fruit, berries or perhaps a light sorbet
If you struggle to resist something sweet; order one dessert for the whole table & share it (you might want to savour the taste)
Other options are to enjoy a nice cup or coffee, cappuccino, or herbal tea or a few pieces dark chocolate if available.

**Beverages**

If you drink alcohol, dry red wine appears to offer the best health benefits - when consumed in small amounts
Remember that a 180ml glass of wine, 350ml light beer, or 30ml shot of alcohol, all contain roughly 120 calories. This can quickly add up!
You can choose a non-caloric beverage such as water (still or sparkling), tea or coffee & still enjoy yourself
As a fizzy beverage; try mineral/soda water with a splash of cranberry, orange, grape or elderberry juice etc
# Mediterranean diet shopping planner

## Fruit
- Apples
- Pears
- Oranges
- Satsuma
- Tangerine
- Clementine
- Grapes
- Cherries
- Bananas
- Peaches
- Nectarines
- Plums
- Strawberries
- Raspberries
- Blackberries
- Blackcurrant
- Redcurrant
- Pomegranate
- Kiwi
- Papaya
- Guava
- Mango
- Melon
- Avocados

## Vegetables
- Carrots
- Tomatoes (fresh, tinned, sauce)
- Celery
- Beetroot
- Broccoli
- Cabbage
- Courgette
- Spinach
- Peppers (all colours)
- Aubergine
- Onions
- Garlic
- Leeks
- Olives
- Peas (fresh or frozen)
- Green beans
- Squash (all varieties)
- Pumpkin
- Swede
- Turnip
- Radish
- Lettuce
- Rocket
- Chard
- Mushrooms (all varieties)

## Beans/Pulses
- Chickpeas
- Lentils (all varieties)
- White (Cannellini) bean
- Black beans
- Black-eyed bean
- Pinto
- Kidney bean
- Aduki bean
- Butter (Lima) bean
- Borlotti bean
- Soy bean
- Tofu or Tempeh
- Hummus

## Wholegrains/Wheat
- Brown rice & flour
- Barley
- Oats or Oatmeal
- Rye
- Spelt
- Quinoa (keen-wah)
- Millet
- Buckwheat & flour
- Whole-wheat bread, Pitta, Pasta
- Whole-wheat flour
- Wholegrain crackers
- Wholegrain bulgur-wheat

## Nuts/seeds
### Unsalted
- Almond
- Brazil
- Cashew
- Hazelnut
- Pine nuts
- Pistachio
- Walnuts
- Sunflower seed
- Pumpkin seed
- Sesame
- Tahini (sesame seed butter)
- Flaxseed
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<th>Herbs/Spices</th>
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<tr>
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<td>Vegetable Bouillon</td>
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| Oils/Fats            |                                 |
|----------------------|                                 |
| Extra virgin Olive oil|                                 |
| Flax-seed oil        |                                 |
| Virgin Rapeseed oil  |                                 |
| Fish oil capsules    |                                 |

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<tr>
<th>Red Wine</th>
<th>Eggs/Milk</th>
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<tr>
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<td>Free range eggs</td>
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<td>Low fat milk</td>
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<td>Soy, Rice, Almond, hazelnut milk</td>
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<td>Low fat unsweetened yogurt</td>
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<td>Low fat cheese</td>
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| Poultry/Meat         |                                 |
|----------------------|                                 |
| Poultry (de-skinned) |                                 |
| Red meat (lean cuts) |                                 |
| Game (much healthier & low in saturated fat) | |
Appendix J. Contents of low-fat diet information booklet

The low-fat diet group received the same foundational advice as the Mediterranean group, but instead of a more comprehensive booklet, they were provided with a low-fat cooking book.

A low-fat cooking booklet contained the following information:

- Low-fat cover sheet
- Lifestyle leaflet
- Healthy shopping tips
- Fruit and vegetable preparation
- Fish and shellfish preparation
- Low-fat cooking: hints and tips
- Snack ideas
- Beverages
- Dining out: hints and tips (see below as different to MeDiet)
- Low-fat shopping list (see below as different to MeDiet)
Low fat cooking: hints & tips

- Remember, that in many cases, your favourite recipes can be modified to reduce their fat content
- Low-fat cooking starts when your shopping
- Use non-stick cookware; it will allow you to cook with little or no oil.
- Measure your oil out first with a teaspoon rather than pouring straight from the container. It will make it easier to control the amount you use.
- A spray or pump bottle is a handy way to reduce your cooking oil use
- Sautéing onions & garlic in a non-stick pan & sealed lid will allow you to create a tasty base for many meals; it must be done with gentle heating, to soften & cook without burning.
- Another tip for sautéing is to use a little white wine, chicken stock, lemon/lime juice or vinegar to add taste at the expense of oil or fat.
- Limit fat-rich fast foods, chips/fries, crisps, salted nuts, pastries, samosas & pies
- Purchase reduced or low-fat dairy options wherever possible; use skimmed or semi-skimmed milk & low-fat cheese & yogurt
- If purchasing meat, buy the leanest cuts with minimal visible fat
- Remove visible fat from meat before cooking: including chicken skin
- Avoid processed meats such as sausages or bacon, which are high in saturated fat
- When eating out, ask if low-fat options are available, or for visible fat to be removed
- Limit high-fat spreads such as margarine & butter by packing your sandwich with tasty fillings such as salad & vegetables
- Instead of using butter & margarine, try spreading your bread or toast with hummus, low-fat cheese spread, mashed avocado or nut spreads such as peanut butter or tahini
- When making low fat soups, curries or casseroles, spoon off excess fat. This is often easier if eating the following day, or when cooled, as the fat will rise to the top and can be easily skimmed off
- Incorporate a wide range of herbs & spices into your cooking. They are an essential way of adding flavour at the expense of salt and fat. Get into the habit of tasting as you cook to ensure you adjust the flavour to suit your taste
- Aim to incorporate alternative cooking methods instead of frying.
  1. Steaming – adds crunch & retains vital nutrients
  2. Boiling – use as little water as possible & don’t over-boil
  3. Poaching – you could try adding vegetable stock or herbs to the water to flavour chicken or fish
  4. Roasting – slow roasting will add sweetness to root vegetables
  5. Tin-foil packets/parcels – a great way to cook fish/chicken with vegetables. Best of all, there’s little to clean up when you finish cooking
  6. Grilling – will help any fat drain from the food, or add taste to vegetables
  7. Stir-frying – cooks vegetables quickly & leaves a crunchy taste, just remember to lightly coat the pan with oil & cut the food into thin, even strips before cooking
# Low-fat diet shopping planner

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<thead>
<tr>
<th>Fruit</th>
<th>Beans/Pulses</th>
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<tbody>
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<td>Apples</td>
<td>Chickpeas</td>
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<td></td>
</tr>
<tr>
<td>Turmeric</td>
<td></td>
</tr>
<tr>
<td>Wasabi</td>
<td></td>
</tr>
<tr>
<td>Vegetable Bouillon</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eggs/Milk</th>
<th>Poultry/Meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free range eggs</td>
<td>Chicken (de-skinned)</td>
</tr>
<tr>
<td>Low fat milk</td>
<td>Turkey (de-skinned)</td>
</tr>
<tr>
<td>Soy, Rice, Almond, hazelnut milk</td>
<td>Duck (de-skinned)</td>
</tr>
<tr>
<td>Low fat unsweetened yogurt</td>
<td>Red meat (lean cuts)</td>
</tr>
<tr>
<td>Low fat cheese</td>
<td>Pork (fat removed)</td>
</tr>
<tr>
<td></td>
<td>Lamb (fat removed)</td>
</tr>
<tr>
<td></td>
<td>Game (much healthier &amp; low in saturated fat)</td>
</tr>
</tbody>
</table>
Appendix K. Low-fat cookbook

Red Beet and Rucula Salad

The beauty of the humble beet is best brought out by roasting them whole, bringing out their naturally sweet and earthy flavor. Don’t let the thought of consuming them intimidate you; it’s not difficult when baking a universe.

Preheat oven to 200 degrees.

Scrub and dry beets and roast them until tender about 45-55 minutes depending on size.

When the roasted beets are cool enough to handle, peel and cut them into wedges.

In a serving bowl large enough to fit everything, whisk the orange juice with the olive oil and salt.

Add the remaining ingredients and toss to combine. Serve immediately before the vegetables wilt.

Serves 4

300 g Red Beets, cut into wedges
1 Orange, zest, finely grated and freshened
1 tablespoon Orange Juice
1 tablespoon Olive Oil
1/2 teaspoon Salt

Summer Oat Salad

We substitute whole-wheat pasta for quinoa in this light and colorful salad. Because it should be served nice and hot, it is perfect to put together in the morning and packed up for a picnic while out on a long walk.

Combine all ingredients except the lettuce and pumpkin seeds and place in the cooler for at least one hour to allow all flavors to combine. Top with dressed salad and spinach just before serving and sprinkle with seeds just before the baby.

Serves 4

300 g Whole Oats or other whole-grain, cooked and cooled
1 Yellow Bell Pepper, chopped
200 g Tomatoes, chopped
1 Carrot, peeled and shredded
1 Leek, washed and finely chopped
2 tablespoons Parsley, chopped
1 clove Garlic, chopped
1 teaspoon Salt
1/2 teaspoon Pepper
Juice from 1 Lemon or Lime
2 tablespoons Capers
100 g Spinach, washed
2 tablespoons Pumpkin or Sesame Seeds

Chickpea Vegetable Curry

This one is best made a day or two ahead. Serve with fresh vegetables and chickpeas. Serve with a dollop of yogurt.

Prep and chop the garlic, ginger, onion, mustard, carrots, and red bell pepper. Heat in a pot over medium heat and add vegetables and cook until tender about 12-15 minutes. Add curry powder and cook until fragrant about 2 more minutes. Add tomatoes, coconut milk, cooked chickpeas, salt and pepper and simmer for 10 minutes.

Serves 4

1 teaspoon Ground Cumin
2 tablespoons Curry Powder
1 teaspoon Dried Red Bell Pepper
1 tablespoon Sesame Seeds
1/2 teaspoon Salt
1/2 teaspoon Black Pepper

2 tablespoons Coconut Milk

2 tablespoons Curry Powder
1 teaspoon Dried Red Bell Pepper
2 tablespoons Sesame Seeds
1/2 teaspoon Salt
1/2 teaspoon Black Pepper

2 tablespoons Coconut Milk
Appendix L. Mediterranean diet PowerPoint slides

AMEND-IT Diet Study

Repair & renewal
- Tissue injury & cell repair is an unconscious process

50-70 billion damaged cells are removed daily
- Food provides the building-blocks for constructing new cells

Inflammation
- Acute inflammation
- Chronic inflammation

Western diet
- Modern farming, harvesting & food processing
- Widespread changes to refined and processed foods
- Chronic illness & allergy
Healthy diet?
- What defines a healthy diet?
- Isolated versus...
- Long-lived populations

Digestion & Energy
- Digesting food requires energy
- Waste products are produced & removed
- Excessive intake of calorie-rich foods leaves little time for repair

Inflammatory foods
- White food products
- Fat including oil drinks
- Processed & partially hydrogenated oils
- Saturated fat & fried foods
- Cooking oils & condensed milk
- Processed & red meat
- Alcohol (and small amounts of red wine)
- Artificial food additives (e.g., preservatives, MSG)

“Calorie-dense, not nutrient-rich”
- We evolved to seek out calorie-laden foods (fats & sugars)
- Sweet, fatty, & salty tastes are addictive
- Cell repair & calorie-dense foods

Spot the difference?

Unrefined plant foods vs. Heart disease & cancer

235
Oxidative stress
- UV light, smoking, pollution
- Physical trauma, another and upset immune system
- Cell damage: illness & aging

Insulin & Glycemic Index (GI)
- [Graph showing GI levels]

Plant protection

Dietary Fats
- All fats are not created equal
- Saturated fat: 
- Monounsaturated fat: 
- Essential fatty acids: Omega-3 & Omega-6

Antioxidants

Dietary Fibre
- [Images of dietary fibre]
  - Helpful bacteria & digestion
  - Prebiotics & a healthy gut
  - Antibiotic therapy
  - Soluble fibre
  - Insoluble fibre
  - Cholesterol reduction
Foundations of a Mediterranean Diet

Juicing

• Vegetables & fruits
• Nutrient-rich
• Antioxidant-rich
• Easy to drink when appetite low

Frozen versus Fresh

• Both & nutrients deteriorate
• Buy fresh when in season
• Frozen produce is generally harvested when fully ripe

Beverages

• Water
• Green & white tea
• Herbal teas, fennel, chamomile, mint, fruit, nettle, ginger
• Soda water & fruit juice (spritzer)
• Sugar & sweeteners

Vitamins & Minerals

Essential building blocks for health

• Water-soluble:
• A, C, D, E, K

• Fat-soluble:

• Enzymes:
1. Chemical reaction needed only
2. Produced by your body or obtained from whole foods

Dining out

• Dining out has increased considerably
• Planning ahead?
• Ingredients & preparation – “just ask!”
• Roman rice
• Share a starter or main course
• Healthier option or cooking methods?
• Ask for left-overs to be removed from the table
• Ask for fat to be removed
• Share a dessert
Final word

- Try new foods & recipes each week
- Vibrant colours
- Be aware of your food cravings
- Portion sizes
- Chew food properly

from 🍎 ➔ 🍓 = 20 minutes

- Be kind to yourself - change takes time
Appendix M. Low-fat diet PowerPoint slides

AMEND-IT Diet Study

Repair & renewal
- Tissue injury & cell repair is an unconscious process

Food provides the building blocks for constructing new cells
- 50-70 billion damaged cells are removed daily

Inflammation
- Acute inflammation
- Chronic inflammation

Western diet
- Modern farming, harvesting & food processing
- Widespread change to refined and processed foods
- Chronic illness & allergy
Healthy diet?
- What defines a healthy...
- Isolated versus...
- Long-lived populations

Digestion & Energy
- Digesting food requires energy
- Waste products are produced & removed
- Excessive intake of calorie-rich foods leaves little time for repair

Inflammatory foods
- White-refined grains & products
- Sugar (including artificial)
- Trans-fats (hydrogenated & partially hydrogenated oils)
- Saturated fat & fried foods
- Cooking oils & Omega-6 fat
- Processed & red meat
- Alcohol (not small amounts of red wine)
- Artificial food additives (e.g., msg, monosodium glutamate)

“Calorie-dense, not nutrient-rich”
- We evolved to seek out calorie-laden foods (food & famine)
- Sweet, fatty & salty tastes are addictive
- Cell repair & calorie-dense foods

Spot the difference?

Unrefined plant foods vs. Heart disease & cancer

World Health Organization (WHO) chart
- Percentage of deaths from heart disease vs. cancer
- Effect of plant foods on heart disease rates

240
Low-fat plant diet

Lean & keen
- Remove skin from poultry
- White fish is naturally lean
- Choose lean red meat cuts & trim visible fat
- Low-fat milk, yogurt & cheese options are widely available

Oxidative stress & Antioxidants

Plant protection

Cooking with vegetable oil
- Heating damages vegetable oil
- Damaged/oxidised oils trigger inflammation
- Lining is 1 cell thick!
- Use a teaspoon to pre-measure (or pump/screw bottle)

Antioxidants
**Insulin & Glycemic Index (GI)**

- Graph showing the glycemic index over time.

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**Vitamins & Minerals**

- Essential building blocks for health
- Water-soluble:
  - B1, B2, B12, C
- Fat-soluble:
  - A, D, E, K
- Enzymes:
  1. Produced by your body or obtained from whole foods
  2. Control biochemical reactions in our cells

---

**Dietary Fibre**

- Helps bacteria & evolution
- Pre-biotic & a healthy gut
- Antibiotic therapy
- Soluble fibre
- Insoluble fibre
- Cholesterol reduction

---

**Juicing**

- Vegetables & fruits:
- Nutrient-rich
- Antioxidant-rich
- Easy to drink when appetite low

---

**Frozen versus Fresh**

- Both fresh & frozen nutrients deteriorate
- Buy fresh when in season
- Frozen produce is generally harvested when fully ripe

---

**Beverages**

- Water
- Green & white tea
- Herbal teas, fennel, chamomile, mint, fruit, nettle, ginger
- Soda water & fruit juice (spritzer)
- Sugar & sweeteners
Dining out

- Dining out has increased considerably
- Plan ahead?
- Ingredients & preparation – “just ask!”
- Portion size
- Share a starter or main course
- Healthier option or cooking methods?
- Ask for leftovers to be removed from the table
- Ask for fat to be removed
- Share a dessert

Final word

- Try new foods & recipes each week
- Vibrant colours
- Be aware of your food cravings
- Portion sizes
- Chew food properly

\[ \text{from} \quad \text{from} \quad \text{to} \quad \text{to} \quad = 20 \text{ minutes} \]

- Be kind to yourself - change takes time
Appendix N. Statistical review

I have provided the following statistical information relating to the above study:

Power calculation

With 40 patients allocated to the ‘MeDiet group and 40 patients allocated to the ‘non MeDiet’ group, then for continuous outcome measures the study will have 80% power to detect effect sizes of 0.6 or between the groups (using a simple two-tailed t-test with the conventional 5% significance level for comparisons between groups at a single time point).

An effect size in this context is the mean difference between the two groups divided by the pooled standard deviation, and an effect size of 0.6 is considered to be a ‘medium’ effect size – Cohen J. (1988). Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum Associates.

Randomisation

Patients will be allocated to one of the two groups using a computer generated block randomization process, stratified by type of transplant, by an individual independent of the study.

Statistical Analysis

Descriptive summary statistics (means, standard deviations, medians, ranges or percentages) will be derived for all measures, and the distribution of the continuous measures will be assessed. Normalising transformations will be used if appropriate.

Group comparisons of all continuous outcomes which follow a Normal distribution (or can be suitably transformed) will be carried out using simple analyses of covariance on the primary endpoint of 52 weeks (adjusting for baseline) and by longitudinal linear regression analysis incorporating data collected at all time points, that is, at baseline, 6, 25 and 52 weeks. These regression models will also include other confounding/predictor variables as required.

Non-normal data will be analysed as change data using the Mann-Whitney U-test.

Yours sincerely

Julie Morris

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Honorary Reader in Medical Statistics
University of Manchester