



## **‘BEST FOODS FOR YOUR HEART’:**

**A RANDOMIZED CONTROLLED PILOT STUDY TO ASSESS THE FEASIBILITY  
OF A MEDITERRANEAN PORTFOLIO DIETARY INTERVENTION FOR  
CARDIOVASCULAR RISK REDUCTION IN HIV DYSLIPIDAEMIA**

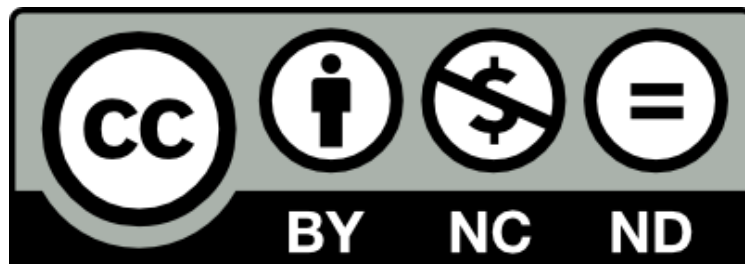
By Clare Stradling

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for the degree of  
DOCTOR OF PHILOSOPHY

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College of Medical and Dental Sciences  
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## ABSTRACT

Portfolio foods (plant stanols, oats, soya, nuts) reduce levels of LDL-cholesterol; the Mediterranean diet reduces risk of cardiovascular disease. The dietary effects are unknown in HIV with the additional burdens of infection, inflammation and antiretroviral treatment (ART). This thesis examines the feasibility and efficacy of the combined Mediterranean-Portfolio diet to reduce cardiovascular risk in people with HIV dyslipidaemia.

Using a pilot, parallel, randomized controlled trial (ISRCTN32090191) recruiting 60 HIV-infected adults on ART with LDL-cholesterol  $>3\text{mmol/l}$ , we observed that advice to follow the Mediterranean-Portfolio diet produced a greater improvement in diet quality, blood pressure, and a significantly greater 10% reduction in LDL-cholesterol at month-6 than standard guidelines to reduce saturated fat intake. Analysis assuming full compliance and preserving randomisation suggests a possible doubling of this estimated treatment effect. Meta-analysis suggests this LDL-cholesterol reduction could reduce major vascular events by 12%.

Process evaluation explored fidelity, dose, uptake and reach, revealing:

- minor adaptations to intervention to accommodate individual needs,
- highly variable adherence (mean  $59\pm 21\%$ ), influenced by socio-economic status and confidence level rather than contextual factors,
- study food intake significantly increased in Mediterranean-Portfolio group,
- positive response to intervention, not intrusive to daily living, with indications of long-term behaviour change.

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## ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral treatment
BFF	Best Foods For your heart
BHIVA	British HIV Association
BMI	body mass index
CD4	cluster of differentiation 4
CI	confidence interval
CT	computerised tomography
CVD	cardiovascular disease
CVE	cardiovascular events
CVR	cardiovascular risk
DEXA	dual energy x-ray absorptiometry
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HOMA	homeostasis model assessment
HR	Hazard Ratio
hs-CRP	high-sensitivity C-reactive protein
IL-6	interleukin-6
LDL	low-density lipoprotein
LMIC	low and middle-income countries
MI	myocardial infarction
MRC	Medical Research Council
NCEP	National Cholesterol Education Program
P	probability value
RCT	randomised controlled trial
RR	relative risk
SD	standard deviation
SMD	standardised mean difference
UK	United Kingdom
US	United States
VL	viral load
WHO	World Health Organisation
WMD	weighted mean difference

# CHAPTER 1: INTRODUCTION

## Background: HIV infection

### Historical context of HIV

Human Immunodeficiency Virus (HIV) probably originated in the 1920s but was not identified as a new retrovirus until 1983 and classified in 1986. Prior to that, several cases of severe immune deficiency such as Kaposi's Sarcoma and Pneumocystis carinii pneumonia were reported in America leading to the creation of the term 'AIDS' (Acquired Immune Deficiency Syndrome) by the Centers for Disease Control (1982). This term served to encompass the collection of opportunistic infections occurring following loss of the body's cellular immunity (Centers for Disease Control 1982). A more in-depth coverage of the historical context of HIV has been written by the author in *Advancing Dietetics and Clinical Nutrition*, Chapter 14 p177-180 (Stradling 2010).

Zidovudine (AZT), was the first antiretroviral drug approved for treatment for HIV in 1987, but effective treatment, with suppression of the virus using three antiretroviral agents (ART), was not possible until the development of new drug classes in the late 1990s. These early ART combinations dramatically decreased death related to HIV infection but were accompanied by new toxicities ranging from nausea and diarrhoea, to dyslipidaemia and lipodystrophy. The story of lipodystrophy from its early definitions, causes, management of lipoatrophy and fat accumulation, development of our current understanding and links with metabolic syndrome can be found in previous work by the author, Chapter 14 p184-192 (Stradling 2010).

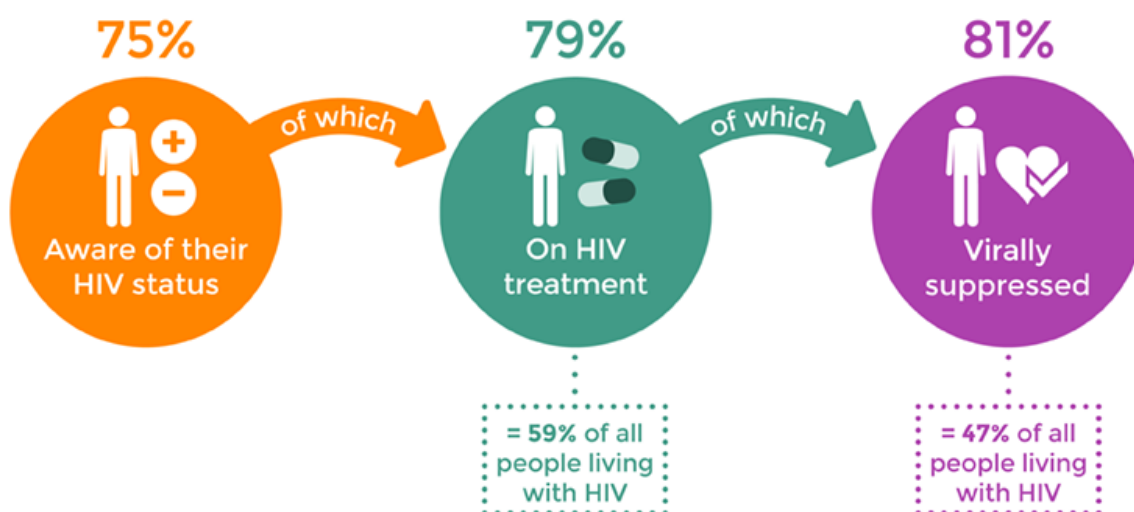
Such was the wide variation in availability of this highly effective ART that it was nearly two decades before the majority of people eligible for treatment received it. Global scale-up of treatment programmes were promoted with ambitious 90-90-90 targets by 2020: 90% of people living with HIV to be diagnosed; 90% of whom to be accessing treatment; 90% of whom achieving viral suppression (UNAIDS 2014). Progress towards these goals is shown in Figure 1. The impact of achievement of these goals has recently emerged with evidence that people who had adhered to treatment and achieved an undetectable viral load did not pass the virus on, prompting the launch of the 'Undetectable = Untransmittable' (U=U) campaign in 2017.

## HIV prevalence

In the United Kingdom (UK) the number of new HIV diagnoses have declined dramatically in the last 2 years. Factors contributing to this success include large increases in HIV testing, improvements in uptake of ART, and scale-up of pre-exposure prophylaxis (PrEP). However, the proportion of people diagnosed at a late stage of infection (CD4 <350 cells/mm<sup>3</sup>) has remained high (41%) and the number of people receiving HIV-related care has increased to 93,385 in 2017 (Public Health England and the Food Standards Agency 2018).

An estimated 36.9 million people were living with HIV globally in 2017 (95%CI 31.1 to 43.9 million), 25% of whom did not know that they were infected (UNAIDS 2018).

## Global progress towards the 90 90 90 targets 2017 (all ages)



Source: UNAIDS Data 2018

Avert) [www.avert.org](http://www.avert.org)

Figure 1 Global progress towards 90-90-90 targets 2017 (all ages)

Source: Infographic from AVERT, Available from: <https://www.avert.org/global-hiv-and-aids-statistics> based on data from (UNAIDS 2018)

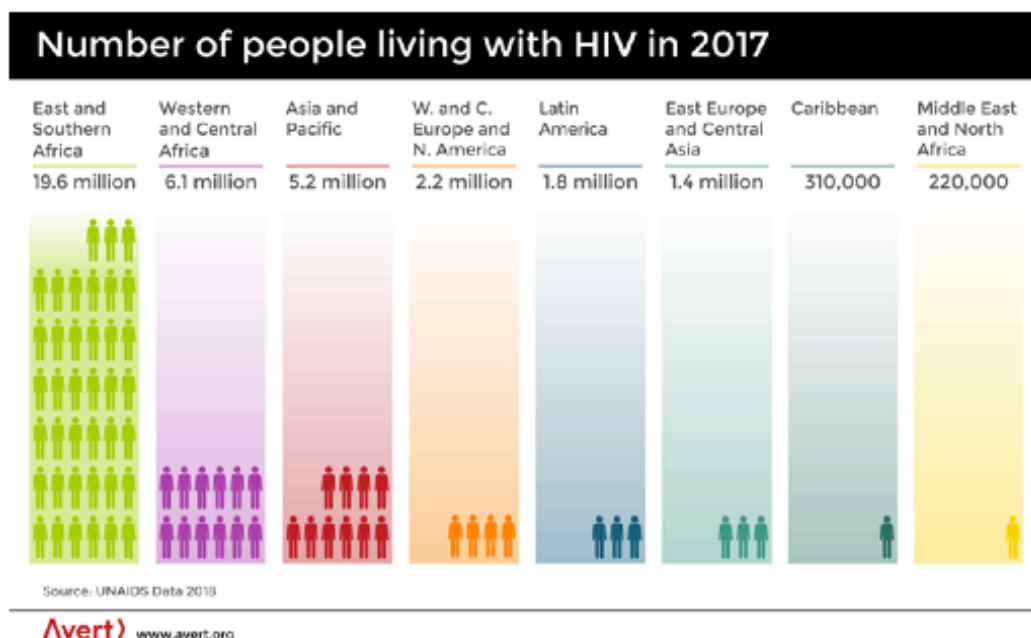


Figure 2 Number of people living with HIV by region

Source: Infographic from Avert (25 September 2018) Available: <https://www.avert.org/infographics/global-no-people-living-hiv-region>



## Current context of HIV; an ageing population

The development of ART has led to substantial reduction in morbidity and mortality for people with HIV infection. Over the past 20 years HIV has transitioned from an advancing terminal illness to a chronic manageable disease, with people living with HIV reaching a life expectancy approaching that of the general population (Antiretroviral Therapy Cohort Collaboration 2017). Despite this triumph, ART does not fully restore health. For those with prolonged HIV infection there remains a higher risk for a number of 'non-HIV conditions' including CVD, cancer, neurocognitive decline, bone, renal and liver disease. These degenerative conditions are similar to those observed among the elderly, hence the suggestion that people with HIV are experiencing premature or accelerated ageing. Evidence to support this theory of premature ageing include studies showing that age-related illnesses such as cognitive impairment, osteoporosis, malignancies and CVD are more prevalent in people with HIV compared to the general population (Guaraldi et al. 2011). They occur at a higher frequency (Schouten et al. 2014), and at a younger age (Guaraldi et al. 2011). Conversely, with this increasing life expectancy, the population of people living with HIV is growing in number and age. As a consequence, a higher prevalence of age-related illnesses would be expected. These comorbidities, however, are higher in those who had been diagnosed >20 years, compared to age-matched people who were diagnosed in later life (Guaraldi et al. 2015).

Potential causes for this proposed premature ageing include inflammation, immune dysfunction and ART. For example, some of the older ART, rarely used now due to their contribution to fat redistribution, also caused mitochondrial toxicity which might

also contribute to cellular ageing. Age-associated changes in immune function are referred to as immunosenescence and share similarities with the association between HIV infection and inflammation, making it difficult to distinguish between the two (Deeks 2011). It is impossible to define the independent effect of HIV infection on ageing, but the consistent observation of a higher risk of comorbidities provides indirect evidence that HIV infection might accelerate the ageing process.

## Cardiovascular disease in HIV

### Overview of cardiovascular disease (CVD)

One of the comorbidities common in HIV is CVD; largely a function of progressive atherosclerosis, which involves deranged lipid metabolism and activation of the innate and adaptive immune systems in the arterial wall. The process of atherosclerosis is akin to an injury response, with the endothelium playing a key role as a biologically active organ. Risk factors for CVD tend to be categorised as non-modifiable (age, gender, race and ethnicity, genetic factors) and modifiable (hypertension, smoking, diabetes, physical inactivity, obesity, high blood cholesterol). In their recent Health Matters campaign, Public Health England changed the emphasis towards behavioural and environmental cardiovascular risk (CVR) factors (see Figure 3) due to the estimated 50-80% of CVD cases that are caused by 'behavioural' risk factors.

### Risk factors of CVD

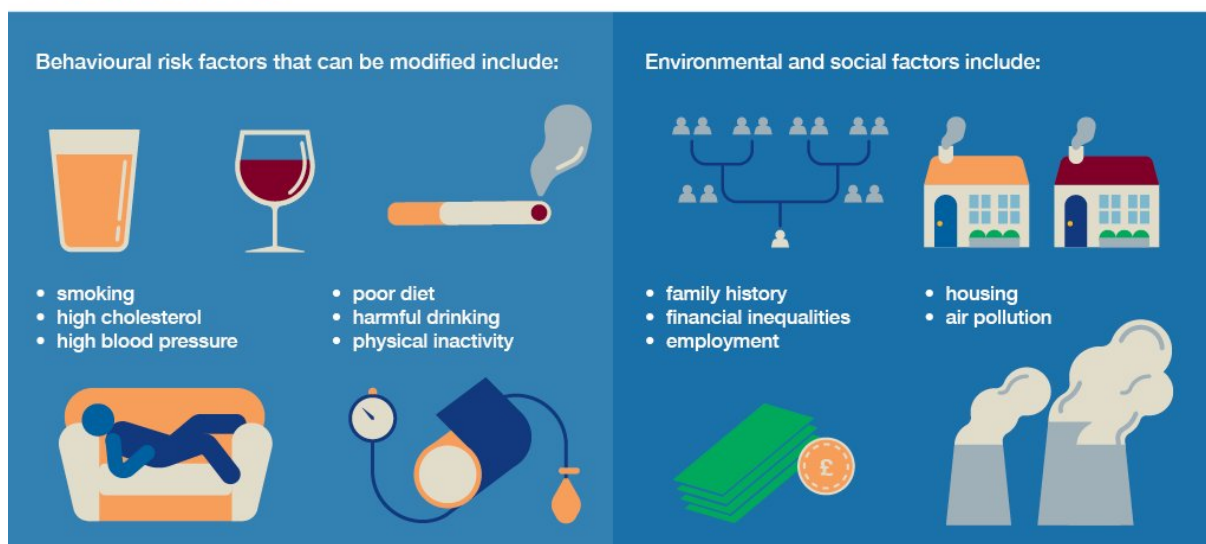


Figure 3 Risk factors for CVD

These 'behavioural' risk factors are greater in lower socio-economic groups, making CVD strongly associated with health inequalities. It can be argued that whilst these behavioural risk factors are modifiable and preventable, they are not completely within the control of the individual due to social factors such as financial limitations, transport systems, fast food, cheap alcohol, tobacco smuggling. Reinforcing a blame culture on the individual's lack of willpower or motivation is counterproductive. An alternative perspective in understanding behaviour and behaviour change is offered by the Improving People's Health strategy (Public Health England 2018) which discusses behavioural and social science disciplines that can inform public health with both upstream and downstream factors. The complexity and overlap of factors influencing the development of a long-term condition such as CVD is illustrated in Figure 4. The CVR factors are numerous and beyond the scope of this thesis, which will focus solely on diet-related CVR factors.

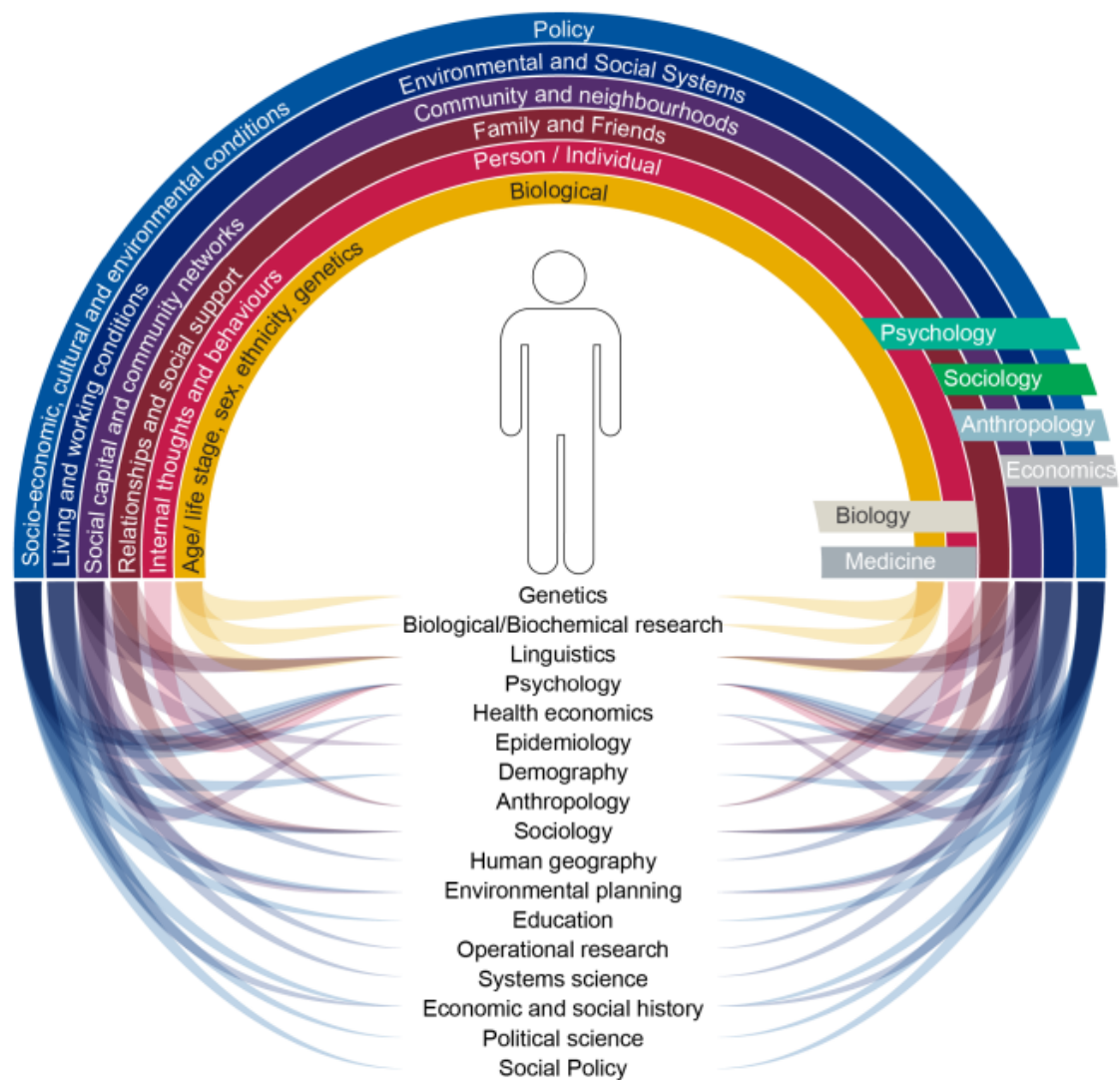


Figure 4 The contributions of behavioural and social science in understanding behaviour and change

Source: (Public Health England 2018) p23

### Increased risk of CVD in HIV

HIV was the cause of 1% of global CVD cases in 2015. The burden of HIV-associated CVD has tripled over the last 20 years with the majority in sub-Saharan Africa and Asia Pacific. National estimates showed that HIV accounted for at least 15% of CVD cases in Swaziland, Botswana, Lesotho and South Africa (Shah et al.

2018). Even in people on optimal treatment with well-controlled HIV (from the international SMART and ESPRIT studies), CVD was found to be the most common cause of non-AIDS related death (Rodger et al. 2013). Closer to home, the leading causes of death in people living with HIV in London during 2016 were CVD, suicide and accidents (Delpech et al. 2018).

Several cohort studies have reported an increased risk of CVD in people with HIV infection, compared to those without HIV (see Figure 5).

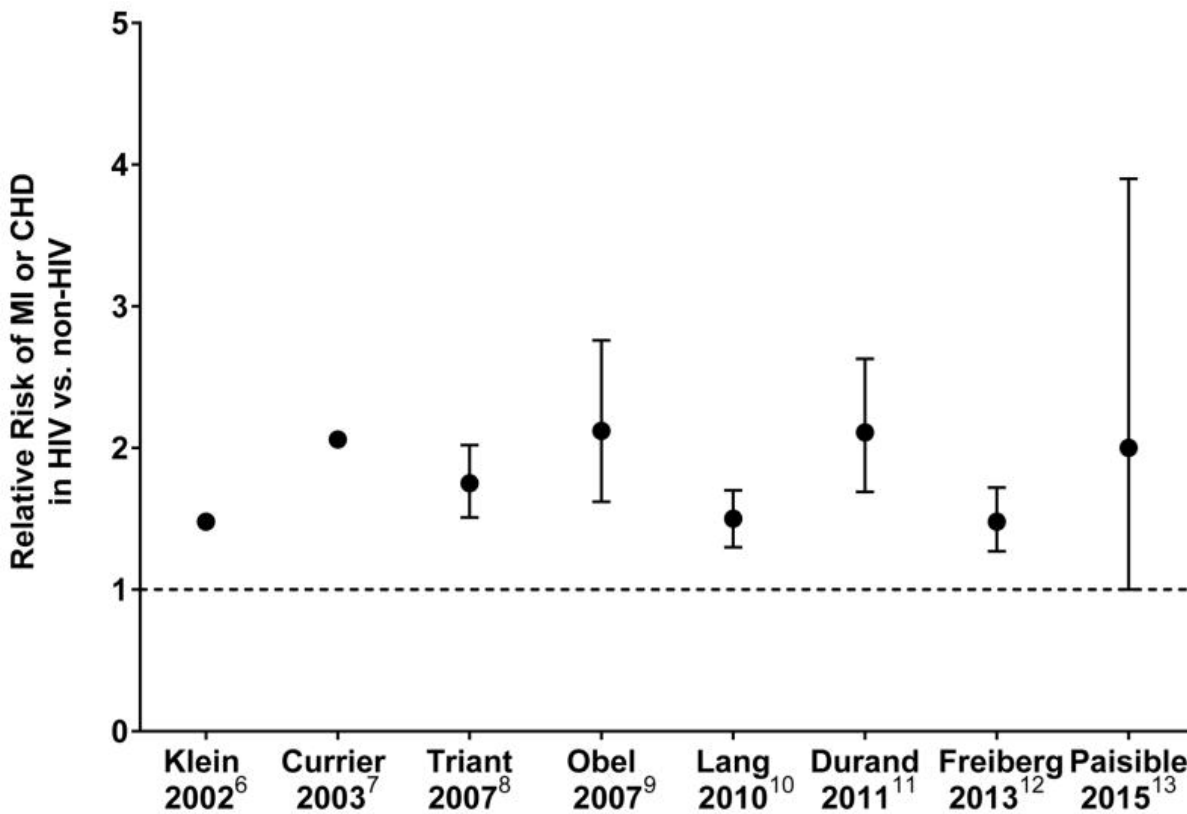


Figure 5 Summary of studies investigating relative risk of CVD in HIV patients versus control subjects.

Data are relative risk with 95%CI where available. Dotted line indicates RR = 1 (relative risk). Source: (Nou et al. 2016) page 2

In deciding whether the increased CVR is real or not, detection of potential differences in the existence and burden of age-related comorbidities in people living with HIV, compared with people without HIV, requires judicious selection of the appropriate uninfected comparison group. These studies commonly use separate cohort studies of uninfected populations or published population rates as the comparator, thereby assuming that the populations are broadly similar and hence, threatening the validity of comparisons. People living with HIV have different demographic, clinical and lifestyle characteristics when compared to uninfected adults in the general population. They are younger, predominantly male, with a higher proportion of minority ethnic groups (Africans in European cohorts, Hispanic/Latinos in US cohorts). There is an increased prevalence of smoking, hypertension, dyslipidaemia, diabetes, substance misuse and hepatitis C, and they are more likely to experience social isolation, food insecurity, and unstable housing (Wong et al. 2014). Many of these differences are risk factors for age-related comorbidities making comparisons subject to epidemiological confounding and therefore threatening the internal validity of the study (Wong et al. 2014). When comparing the two populations some cohort studies adjusted for CVR factors such as hypertension, smoking, diabetes and dyslipidaemia (such as (Triant et al. 2007) Other studies included additional confounders such as race, Hepatitis C, alcohol and cocaine use, whilst some adjusted solely for age and gender (such as (Helleberg et al. 2015)) (Shah et al. 2018) thus ignoring the potential bias introduced by the effects of these factors. One such factor, socio-economic status, is difficult to quantify and yet can have a substantial impact, as seen by significant variation in HIV mortality rates depending on educational status and ethnicity, factors acting as a proxy for

socio-economic status (Simard et al. 2012). Differences between populations need to be accounted for in the design and/or analysis in order to generate valid inferences on the impact of HIV infection and its treatment on CVD.

One study addressed this issue of finding a suitable comparison group by creating a virtual cohort. The Veteran's Ageing Cohort Study is a large ( $n = 82,459$ ), diverse, prospective longitudinal cohort that enrolls military workers with and without HIV from the US Department of Veterans Affairs health care system. Drawing data from a number of linked databases, they match the two groups for the potential confounders of age, ethnicity, and clinical site, and then examine incident myocardial infarction (MI) rates in people with and without HIV, adjusting for Framingham CVR factors, comorbid diseases and substance use during the statistical analysis. They demonstrated that the HIV population experience a 50% increase in MI rates (incident acute MI HR 1.48, 95%CI 1.27 to 1.72) that is not explained by traditional CVR factors and persists in those achieving viral suppression (Freiberg et al. 2013). A limitation of this cohort is that they are predominantly male. Similarly, the Multicenter AIDS Cohort Study (MACS) is all male, but recruiting both infected and uninfected men from Los Angeles, Baltimore, Chicago, Pittsburgh, Jacobson so as to have a representative comparison group (Post et al. 2014). Conversely, the Women's Interagency HIV Study is all female, also recruiting a control cohort from similar cities to MACS, with additional sites on the east of the US (Jackson, Birmingham, Atlanta, Miami, Washington, Baltimore, New York) (Adimora et al. 2018). Combined, these two studies provide a more complete perspective for comparison between cohorts.

Another limitation of these cohort studies was the relatively low event rates, probably due to insufficient follow-up time of a generally young population (Nou et al. 2016). This was recently addressed by the largest meta-analysis to date that pooled data on incidence of CVD from 16 studies, including cohorts from Europe, the US and one LMIC (Low and Middle Income Countries - Tanzania) (as opposed to the 8 studies in Figure 5 on page 8). Compared to uninfected individuals, the risk of CVD was 2-fold higher for people with HIV (RR 2.16, 95%CI 1.68 to 2.77,  $I^2=95\%$ ) (Shah et al. 2018). There is some encouraging evidence from the D:A:D study (11 cohorts from 33 countries in Europe, US and Australia) suggesting that CVD-related deaths may be declining over time, using comparisons between 1999 and 2011 data (Smith et al. 2014). However, this is not reflected in other areas of the world as the global population-attributable fraction from CVD attributable to HIV has increased from 0.36% to 0.92% over the past 26 years (Shah et al. 2018), the burden of which lies mostly in sub-Saharan Africa.

The evidence of an increased risk of CVD in HIV extends to measures demonstrating the presence of subclinical CVD but is not entirely consistent. Non-invasive imaging of the arteries have shown increased carotid intima-media thickness (cIMT) in people with HIV infection compared to uninfected controls (Grunfeld et al. 2009; Hultén et al. 2009), and increased coronary plaque volume measured by coronary computed tomography angiography (Lo et al. 2010). However, measurements of coronary artery calcium (indicating atherosclerotic calcification of the arteries) have shown no significant difference between groups (Hultén et al. 2009). The computed tomography technology allows visualisation of the plaques such that the calcified



plaques (indicative of more advanced stable atherosclerosis, prevalent in people with CVD) can be distinguished from the non-calcified plaques (found to be more prevalent and extensive in people with HIV (Post et al. 2014)) offering a potential explanation for the lack of increased coronary artery calcium. Traditional CVR factors contribute to calcified plaques, whereas monocyte and macrophage activation are associated with non-calcified plaque in people with HIV (Lo & Plutzky 2012). These differences suggest that HIV-specific mechanisms may have a key role in accelerating atherosclerosis via an altered plaque morphology that has a greater propensity to rupture (Zanni et al. 2013). This is consistent with findings from a recent study in two demographically different HIV cohorts showing that carotid artery plaques were predictive of non-HIV related death (HR 1.44, 95% CI 1.10-1.88) in people with HIV (Hanna et al. 2018). No association was found with CVD death, but the study was limited by incomplete information on incident CVD events. The contradictory nature of the evidence relating to presence of subclinical CVD in people with HIV, potentially highlights current lack of understanding on the exact HIV related mechanisms that contribute to CVD.

The recent analysis showing a 2-fold increased risk of CVD in people living with HIV has prompted the suggestion that HIV should be considered a major CVR factor alongside diabetes, hypertension, smoking and raised cholesterol (Hsue & D. D. Waters 2018). Hippisley-Cox et al. (2017) included HIV as one of new potential predictor variables under test in the latest validation update of the QRISK3 CVD prediction algorithms. HIV did not meet the model inclusion criteria as a new risk factor because although the adjusted hazard ratio was greater than 1.10 (HR 1.25 in

women; HR 1.17, 95%CI 1.03 to 1.35 in men) it was not statistically significant at the 0.01 level due to inadequate sample size of people living with HIV (n = 12,064) within the GP cohort (n = 7,889,803) (Hippisley-Cox et al. 2017). The authors acknowledged that with increasing numbers of patients disclosing their HIV diagnosis to their GPs, improved documentation of ART, and the ageing population resulting in higher number of cardiovascular events (CVE), the inclusion of HIV as a risk factor will need to be reviewed at future updates.

### **Aetiology of CVD in HIV**

The underlying mechanism driving excess CVD risk is not clear but probably involves a combination of factors including the burden of traditional CVR factors, the virus itself, side effects of ART, and non-traditional risk factors such as hepatitis C, substance abuse. Recent research has identified plausible biological mechanisms for a direct link between HIV and CVD, including vascular inflammation, dyslipidaemia and insulin resistance.

### **Factors contributing to CVD – Traditional cardiovascular risk factors**

Risk factors for CVD are well-defined within the general population (Figure 3). These traditional CVR factors tend to be more common in the HIV population, such as smoking rates ranging from 35 to 72% (Sabin & Worm 2008). Although the global smoking prevalence has fallen, the prevalence amongst people with HIV remains high and is double that in the general population in the US (42% versus 21%) (Mdodo et al. 2015) and UK (37% (Aboud et al. 2010) versus 15% (Office for National Statistics 2018)). Substantial geographical variation exists with recent

smoking prevalence reports of 27% in LMIC (Mdege et al. 2017), 52% in South Africa (Elf et al. 2018) and 60% in European and North American cohorts (Helleberg et al. 2015). Similarly, rates of diabetes in people with HIV exhibit geographical variation with 3-5% in European cohorts (De Wit et al. 2008) and 11-15% in the US (Triant et al. 2007). A possible explanation might be the difference in obesity rates, a known risk factor for diabetes, as body mass index (BMI) is higher in US studies. Weight itself is a CVR factor but may have more of an impact on the health of people with HIV. This is because weight gained in the first year after ART initiation is associated with greater risk of diabetes than same weight gain in uninfected individuals (Herrin et al. 2016; Achhra et al. 2018). In a separate study for each 5 pounds of weight gained, the risk of diabetes increased by 14% in people with HIV (HR, 1.14; 95% CI, 1.10–1.17), compared to 8% in those without (HR: 1.08; 95% CI, 1.07–1.10;  $p < 0.01$  for interaction). The relationship with CVD may be more complex because although the risk of diabetes increased linearly with increasing BMI, the risk of CVD was found to increase at low ( $<18.5$ ) and high ( $<30$ ) BMI (Achhra et al. 2018). Presence of hypertension has also been found to be higher in people with HIV than those uninfected (21% versus 16%) (Triant et al. 2007), likewise dyslipidaemia (23% versus 18%) (Triant et al. 2007). This evidence for a relatively high prevalence of CVR factors does not adequately account for the 2-fold increased risk of CVD as shown in meta-analyses, as adjustment for these variables were accounted for in the majority of cohorts (Shah et al. 2018).

## Factors contributing to CVD - HIV

Dyslipidaemia has always been a characteristic of HIV infection. Prior to ART treatment the picture was of reduced levels of high-density lipoprotein- (HDL-) cholesterol, that correlated inversely with viral load, potentially reflecting HIV-mediated interruption of the reverse cholesterol transport pathway through which cholesterol is cleared from peripheral tissues (Feeney et al. 2013). Levels of HDL-cholesterol have remained depressed during ART treatment, frequently accompanied by elevated triglyceride levels rather than LDL (low-density lipoprotein) -cholesterol as in the general population. There is limited understanding as to the reasons for diminished HDL-cholesterol, despite it being second to age as the largest contributor to CVR (Duprez et al. 2012). Some studies report increases in HDL-cholesterol on ART, however closer investigation has found HDL particles to be larger, less stable, and bind less readily to hepatocyte receptors than normal HDL-cholesterol (Gillard et al. 2013), raising questions as to their ability to function beneficially. The mechanisms behind HIV-related hypertriglyceridaemia are complex and only partly understood but involve a combination of excessive hydrolysis of free fatty acids, with insufficient oxidation response, resulting in an increased flux of plasma fatty acids, and defective clearance of VLDL-triglyceride (very low density lipoprotein-triglyceride) by adipocytes (Sekhar 2015). In HIV there appears to be a vicious cycle, where inflammation increases lipid levels, promoting modification of their composition and function. These oxidised lipids in turn perpetuate inflammation (Funderburg & Mehta 2016), illustrated by the significant association between inflammatory markers and serum lipids in 181 virologically suppressed patients on ART (Višković et al. 2018).

Persistent immune activation and systemic inflammation plays a role in the development of HIV disease and contributes to the increased risk of CVD. Viral suppression by ART does not completely nullify viral replication, particularly in viral reservoirs, resulting in continued residual inflammation and increased risk of atherosclerosis, even in successfully treated patients (Funderburg & Mehta 2016; Nou et al. 2016). Chronic HIV infection induces endothelial dysfunction which leads to the activation of the inflammatory response and promotion of local thrombosis, important in plaque formation (Višković et al. 2018). The virus and resulting inflammation appear to play a key role from the initial phase to the final lesions of atherosclerosis, evidence for which is presented in Table 1.

#### **Factors contributing to CVD - Antiretroviral treatment**

The role of ART in the development of CVD in people with HIV remains the subject of debate. Since the SMART trial reports of increased CVE (and elevated inflammatory markers IL-6 and D-dimer) in patients receiving intermittent ART (when CD4<350 cells/mm<sup>3</sup>) compared with patients on continuous ART (INSIGHT SMART Study Group 2006), the clinical opinion has been that sustained treatment with ART to reduce HIV viremia and inflammation could be used as a strategy to prevent CVD (Clumeck et al. 2008). Whilst this may be the case for prevention of HIV-related mortality, as shown subsequently by the START trial findings, where initiation of ART in people with CD4>500 cells/mm<sup>3</sup> provided net benefits over starting ART when CD4 counts had declined to 350 (The INSIGHT START Study Group 2015), neither study was powered for a CVD endpoint. The difference between groups in the SMART trial was not statistically different ( $P = 0.05$ ), and post hoc analysis showed that neither

CD4 or viral load was significantly associated with CVE (Phillips et al. 2008). In START, rates of CVE were similar in both arms; but this was a young population with a low CVD risk (median 10-year Framingham risk 1.9%) (The INSIGHT START Study Group 2015).

Some observational studies examining rates of MI in HIV populations have reported increased risk associated with lower CD4 and detectable viral load (Drozd et al. 2017) but overall have failed to show clear CV protective effects for those on ART, with a CD4>350, or suppressed viraemia (Churchill et al. 2016):Table 8.1). This is primarily because the studies have not set out to answer the question of whether ART affects CVE in those who are at high CVR.

Certain ART, such as nucleoside reverse-transcriptase inhibitors and cumulative exposure to some protease inhibitors, have been associated with increased risk of MI (D:A:D Study Group et al. 2008), an effect not fully explained by traditional cardiovascular risk factors. Understanding of the pathogenesis is limited, with many pathways implicated. For example, protease inhibitors such as ritonavir inhibit the proteasomal degradation of apolipoprotein B, causing increased secretion of Apolipoprotein B containing lipoproteins such as triglyceride-rich VLDL (very low density lipoprotein cholesterol) (Leyes et al. 2018). Another protease inhibitor, lopinavir, was found to increase intestinal cholesterol absorption rather than cholesterol synthesis (Leyes et al. 2018). Certain ART can induce dyslipidaemia and therefore may contribute to CVR, such as the nucleoside reverse-transcriptase inhibitor efavirenz increasing levels of total cholesterol and triglycerides (Williams et al. 2009). Collectively, the data suggest that newer ART appear to have less

Table 1 HIV involvement in stages of atherosclerosis

Stage of atherosclerosis	Evidence in HIV population
Adhesion of monocytes to the activated endothelium	Increased markers of endothelial activation (such as soluble intercellular adhesion molecule 1, soluble VCAM-1, and von Willebrand factor) which have been found to reduce with ART, and correlate with viral load (Lo & Plutzky 2012).
Monocytes are activated by microbial translocation and other factors	<p>Gut integrity is impaired early in HIV infection and persists despite ART, leading to translocation of gut microbial and metabolic products into the circulation, such as raised concentrations of lipopolysaccharide (a component of Gram-negative bacteria) which has been associated with progression of coronary intima-media thickness (Kelesidis et al. 2012).</p> <p>Increased levels of oxidised LDL-cholesterol, a precursor to activated monocytes (Funderburg &amp; Mehta 2016).</p>
Monocytes migrate into the intima, maturing into macrophages	Increased monocyte-macrophage activation markers (soluble CD14, soluble CD163) which independently predict all-cause mortality (d'Ettorre et al. 2016).
Macrophages take up oxidized LDL-cholesterol to form foam cells	HIV down regulates ATP-binding cassette transport A1 impairing cholesterol efflux capacity. This hampers reverse cholesterol transport, where cholesterol is removed from macrophages by HDL-cholesterol, protecting against plaque development. (Nou et al. 2016)
Smooth muscle cells migrate to intima, thickening vascular wall producing lesion with fibrous cap	<p>Presence of high-risk, non-calcified plaques that are susceptible to rupture (Post et al. 2014).</p> <p>Pro-inflammatory diet increases levels of cytokines (IL-1 and TNF-alpha) which attract inflammatory cells and thicken vascular wall (Willerson &amp; Ridker 2004).</p>
Signalling between T cells and macrophages promotes release of matrix-degrading enzymes which destabilise plaque leading to rupture (Lo & Plutzky 2012)	Increased levels of pro-inflammatory cytokines (C-reactive protein CRP, D-dimer, interleukin-6 IL-6) in those who experienced more CVE in the SMART study {Duprez:2012je} and a number of case control studies (Vos et al. 2016).

metabolic effects than with older regimens, resulting in a potentially greater net benefit with respect to CVR. However, this assumption that contemporary protease inhibitors are better may in time prove to be false, with the emergence of more data such as the recent analysis from the D:A:D cohort (n = 35,711) suggesting that cumulative use of darunavir, but not atazanavir, is associated with progressively increasing risk of CVD (Ryom, Lundgren, et al. 2018).

### **Factors contributing to CVD - Summary**

The amplified risk of CVD has been attributed to intersecting epidemics of an ageing HIV cohort, continued inflammation and immune dysfunction despite successful ART, increased prevalence of dyslipidaemia, physical inactivity and smoking. HIV-related factors may only partially be assuaged by ART and may be aggravated by effects not fully explained by conventional CVD risk factors. Given that the understanding of the pathogenesis is limited, it would be naïve to presume that interventions to reduce CVR in general population will translate to similar reductions in MI incidence in HIV populations (Mallon 2013). It is therefore essential that the effectiveness of dietary interventions, such as the Mediterranean diet, are tested in people living with HIV.

### **Management of CVD within HIV guidelines**

Most of the HIV guidelines have a section on management of comorbidities that advocates assessment and management of CVR for all individuals with HIV, specifically targeting those >40 years, in order to determine those at increased risk who require intervention. Definition of 'high risk' varies geographically depending on



the resources available for implementation, >10% UK (Churchill et al. 2016), >20% Europe (Ryom, Boesecke, et al. 2018), >30% LMIC (World Health Organisation 2016).

British (Churchill et al. 2016) and European (European AIDS Clinical Society 2017) guidelines concur with the good practice statement from WHO (World Health Organisation 2016):p216, 'Strategies for the prevention and risk reduction of CVD by addressing modifiable factors such as high blood pressure, smoking, obesity, unhealthy diet and lack of physical activity should be applied to all people living with HIV.' In general, the HIV guidelines lack detail, deferring to national CVD prevention guidelines for direction on the implementation of modification of lifestyle factors and rationale.

Within the HIV guidelines, management of CVR has historically focused on switching ART to those with a more favourable metabolic profile (Churchill et al. 2016). The British HIV Association (BHIVA) guidelines assert the caveat that although the aim of switching is to improve dyslipidaemia, there is no clinical trial evidence that interventions to amend plasma lipids will reduce CVD risk in the context of HIV disease. Examining ART switch studies detailed in table 8.3 of BHIVA guidelines (Churchill et al. 2016)p124 reproduced in Appendix, indicates that significant reductions in LDL-cholesterol in the region of -0.2 to -0.4mmol/l (5 studies) were achieved by switching one drug to another. A larger effect was observed in a pilot study switching from efavirenz to etravirine with secondary analysis from 22 patients using Wilcoxon signed-rank test to detect LDL-cholesterol reduction of -0.6  $\pm$ 1.1mmol/l, P = 0.02 (L. Waters et al. 2011). Extreme caution is required with

interpretation as ART switch studies are not usually powered for comparisons in lipid levels, and cross-study comparisons are not possible due to differences in populations, length of follow up and baseline lipids. However, given that these switch studies form the backbone of medical management of CVD prevention in HIV, any future evidence of a similar reduction in LDL-cholesterol from dietary interventions should be given equal prominence.

HIV is gradually being acknowledged in the mainstream CVD guidelines. The European Society of Cardiology guidelines now recommend lipid lowering therapy to reduce LDL-cholesterol to  $<1.8\text{mmol/l}$  in individuals with HIV (Piepoli et al. 2016) acknowledging that whilst dietary changes, regular physical activity and switching to another ART regimen may improve dyslipidaemia, most patients still need pharmacological therapy to reach lipid goals. Statins are recommended, although those metabolized in the liver via the CYP3A4 or CYP2C9 can interact with protease inhibitors and efavirenz, so should be avoided. The potential for drug interactions should be checked using the University of Liverpool HIV drug interaction database (<http://www.hiv-druginteractions.org>). Despite these recommendations the prescription of statins in people with HIV remains suboptimal, as reports suggest that only 50% of patients requiring statins are properly treated (De Socio et al. 2016). With the increased incidence of insulin resistance in people living with HIV (Grunfeld et al. 2007), caution should be exercised with the use of statins due to the increased risk of incident diabetes with statin use (9% risk (Sattar et al. 2010), 25% in JUPITER trial (Ridker et al. 2012), 14% per year of use (K. A. Lichtenstein et al. 2015)). There is no data on the effects of statins, fibrates or ezetimibe on CVE in people with HIV

dyslipidaemia, although the REPREIVE trial are currently recruiting to address this (Gilbert et al. 2015).

## Review of the literature: Diet for prevention of CVD

### Dietary nutrients

Diet is one of the key factors that can be modified to prevent and delay the progression of CVD. The global Burden of Disease study found that the leading cause of years of life lost in England in 2013 was attributable to CVD. The most prominent behavioural risk factor was suboptimal diet, being the largest contributor to disability life adjusted years (10.8%, 95% uncertainty interval 9.1 to 12.7) (Newton et al. 2015). Epidemiological studies examining the relationship between diet and disease related death have traditionally focused on the analysis of single nutrients or foods. The evidence on nutrition and CV health with regards to 10 specific foods and nutrients (fats, fish, nuts, fruit and vegetables, soy protein, carbohydrates, wholegrains, dietary fibre, electrolytes, micronutrients) has previously been presented by the author in a 22-page paper '*A review of dietary influences on cardiovascular health, part 1: the role of dietary nutrients*' (Stradling et al. 2013). Key evidence that has emerged since that publication will be discussed briefly here.

### Vitamins

Further evidence from randomised controlled trials (RCTs) have helped to clarify issues around vitamins. A recent meta-analysis supports previous conclusions from Stradling et al (2013) that data on multivitamin supplements, vitamin C, calcium,

vitamin D show no consistent benefit for the prevention of CVD (Jenkins et al. 2018). The previous concerns about obtaining antioxidants from supplements rather than foods have been substantiated by data showing that antioxidants were associated with an increased risk of all-cause mortality (RR 1.09 95%CI 1.04 to 1.13,  $I^2=0\%$ ,  $n=15$  RCTs). Further unease will arise from the new findings that niacin increased all-cause mortality by 10% (RR 1.10 95%CI 1.00 to 1.20,  $I^2=0\%$ ,  $n=3$  RCTs), raising questions as to its suitability as a long-term adjunct to statin therapy.

The B vitamins have previously been of interest due to their association with homocysteine levels, however recent observations that reduction of homocysteine was not associated with stroke reduction (Dong et al. 2015) suggest that an alternative mechanism exists. The same meta-analysis found low quality evidence for preventive benefits of folic acid for total CVD (RR 0.83 95%CI 0.73 to 0.93,  $I^2=0\%$ ,  $n=5$  RCTs), and folic acid with B-vitamins B6 and B12 for stroke (RR 0.90 95%CI 0.81 to 1.00,  $I^2=16\%$ ,  $n=12$  RCTs). However, the folic acid benefits were driven by a Chinese 5-year RCT with 20,700 participants (providing 92% weighting to the meta-analysis) making the generalizability of these findings to other countries limited due to the lack of folic acid fortification in China (Jenkins et al. 2018).

## Fats

Two draft guidelines are currently awaiting publication following public consultation (Scientific Advisory Committee on Nutrition 2018; World Health Organisation 2018). Having reviewed the current evidence both committees are suggesting that the overall existing recommendations will remain unchanged, with total fat consumption

limited to less than 30% of total energy intake, and a continued shift away from saturated towards unsaturated fats.

Despite reviewing 47 systematic reviews on saturated fat, Public Health England still highlighted the inadequacies of the available evidence concerning which fatty acids should be used to replace saturated fat. Lack of differentiation in studies between n-3 and n-6 fatty acids prevented clarification. Stradling et al.'s review (2013) previously highlighted the methodological limitations that beset these studies and analyses, including publication bias, confounding from trans-fat and use of statins, heterogeneity between pooled studies particularly regarding the composition of the diet such as mixed sources of n-3 and n-6 polyunsaturated fatty acids, lack of detail about the type of the carbohydrate (wholegrain or refined), and unspecified replacement nutrients. These limitations were re-iterated in the SACN report with additional concerns about lack of information on statistical power, and studies with short follow-up time restricting the use of CVE as outcomes (Scientific Advisory Committee on Nutrition 2018).

The WHO committee (World Health Organisation 2018) examined additional evidence on the effects of individual saturated fatty acids on the lipid profile. Although the C12-C16 fatty acids (palmitic, myristic, and lauric acids) were observed to exert the strongest negative impact on LDL-cholesterol in one study (MensinkWorld Health Organization 2016), it was concluded that there was insufficient evidence for recommendations to be made. Similarly, differences in health outcomes between ruminant and industrially produced trans fatty acids were observed in another study but were explained as likely to be due to differences in dose rather than type.

### The PURE study

The Prospective Urban Rural Epidemiology (PURE) study is the largest cohort study to be published in the last 5 years, and challenges current dietary recommendations (Teo et al. 2009). Between 2003 and 2013 135,335 participants aged 35-70yrs were enrolled from 18 countries including 3 high income (Sweden, Canada, United Arab Emirates), 11 middle income, and 4 low income. Primary outcomes of total mortality (n = 5,796) and CVE (n = 4,784) were monitored during the 7-year follow-up period. Dietary data from baseline food frequency questionnaires was analysed into quintiles of nutrient intake based on percentage of energy provided by fat, carbohydrate and protein. Higher fat intake was associated with lower risk of total mortality (HR 0.77, 95%CI 0.67 to 0.87) (Dehghan et al. 2017). The highest quintile equated to 35% of total energy intake from fat, which although slightly above UK guidelines of 30%, is consistent with a Mediterranean diet. It could be argued that the findings are concordant with current dietary advice, as the lowest quintile equated to 11% of total energy intake from fat, which is considerably lower than that observed in the UK and North America.

Likewise, higher saturated fat related to lower total mortality HR 0.86 (95%CI 0.76 to 0.99). However, the lowest quintile was used as the reference group in the analysis, and this assumes that the relationship is linear. Results suggested that the relationship for saturated fat may be U-shaped, as the mortality and MI was highest in quintile 1 (where saturated fat contributed 2.8% of energy) before falling, then increasing again in quintile 5 (13.2% of energy). Mean intakes of saturated fat in the US and UK are considerably higher suggesting that the findings of this study may not

be generalisable to high income countries. Whereas current dietary guidelines to reduce saturated fat intake to below 10% (7% in US) are based on studies conducted in North America and European populations where intake of saturated fat is relatively high, and CVD was a major cause of death.

Another finding from this trial suggests that current guidelines focusing on fat reduction rather than carbohydrate may be misleading, as higher carbohydrate intake was associated with increased risk of total mortality (highest quintile vs lowest quintile, HR 1.28, 95%CI 1.12 to 1.46) (Dehghan et al. 2017). However, the type of carbohydrate was not disaggregated, so it was unclear what proportion were refined or wholegrain. Carbohydrates also highlight the issue of confounding, as the top contributor for Bangladesh was white rice, which may reflect a 'poverty' diet as confounders associated with being poor cannot be fully ruled out. The influence of residual confounding in a study of this size and diversity cannot be underestimated. Residual confounding results from large variation in socio-economic factors such as ability to afford or access certain foods, general health status and access to health care that cannot fully be accounted for (Malhotra et al. 2018). Similarly, the effect of different diets could differ depending on the background nutritional status of the population. Lower total death but not CVD death with higher intakes of fat and protein could reflect the need to treat nutritional deficiencies and that adequate nourishment was the reason for less non-CVD death (Gianos et al. 2018).

It is important to acknowledge the role of observational data in providing guidance on relative, not absolute, risks or benefits of nutritional components and avoid oversimplification of 'good' and 'bad' nutrients. Whilst the PURE study provides a

healthy challenge of current thinking, it is important to look towards clinical trial data and the study of dietary patterns, thus avoiding the problem of confounding by specific aspects of the diet

## Dietary patterns

The examination of single nutrients, as presented above, is problematic because nutrients can interact synergistically or antagonistically. In addition, the effect of a single nutrient on any outcome is likely to be very small requiring large sample sizes to detect a difference. Therefore studies have tended to test numerous nutrients raising concern over the statistical issues associated with multiple testing, whereas testing dietary patterns avoids confounding from specific aspects of the diet and increases statistical power (García-Arellano et al. 2018). People also do not eat nutrients in isolation, but rather foods, hence the emergence of dietary patterns as the means to study how foods are consumed in combinations and their relationship with disease. The dietary patterns of the Mediterranean diet, Dietary Approaches to Stop Hypertension (DASH) diet, and Very low carbohydrate diet and their relationship with CVD have been examined in the 13-page second part of the review by the author (Stradling et al. 2014). The following is an update to that paper.

### Portfolio dietary pattern

The Portfolio dietary pattern is a plant-based dietary pattern devised as a 'portfolio' of 4 foods, each with an approved health claim for cholesterol-lowering properties: nuts, soy protein, plant sterols, and soluble fibre ( $\beta$ -glucans in oats, barley, beans, lentils). Following feasibility testing in 2002, this combination of foods was trialled in an



efficacy study where 25 participants with raised LDL-cholesterol levels ( $>4.1\text{mmol/l}$ ) were randomised to either the Portfolio diet (also vegan) or a low saturated fat diet (also lacto-ovo vegetarian, National Cholesterol Education Program NCEP step 2 guidelines) for 4 weeks. Food diaries showed that actual intakes equated to 4-5% of energy from saturated fat in both groups. All foods were provided except for fruit and vegetables. LDL-cholesterol was reduced by  $12.1\pm2.4\%$  on the low-fat diet and  $35.0\pm3.1\%$  ( $-1.59\text{mmol/l}$ ) on the Portfolio diet (Jenkins et al. 2003). Further studies were then undertaken with free-living participants and extended durations. A recent meta-analysis combined data from all of the 7 Portfolio studies conducted by the Jenkins group ( $n = 439$ ) showing reduction in LDL-cholesterol of  $-17\%$  (MD  $-0.73$ , 95%CI  $-0.89$  to  $-0.56\text{mmol/l}$ ,  $I^2=67\%$ , 7 RCTs) (Chiavaroli et al. 2018). The degree of LDL-cholesterol reduction varied between study design, with  $-21\%$  in efficacy studies and  $-12\%$  in effectiveness trials (Chiavaroli et al. 2018). Adherence was found to be directly associated with reductions in LDL-cholesterol, with different levels of adherence observed in efficacy trials  $>90\%$  (Jenkins et al. 2006) and effectiveness trials  $<50\%$  (Jenkins et al. 2011).

### **Mediterranean diet**

The Mediterranean diet can be described as a plant-based diet that allows for a low consumption of meat, fermented dairy and a moderate consumption of fish (Trichopoulou et al. 2014). Consistent with the findings of the secondary prevention Lyon Diet Heart study (de Lorgeril et al. 1999) and the 5-year PREDIMED primary prevention trial (Estruch et al. 2018), a number of long-term observational studies have supported protective roles of the Mediterranean diet against CVD. These

studies have been discussed in detail in a previous review on dietary patterns (Stradling et al. 2014):p51-55). Publications since that date have included meta-analyses, the findings of which vary depending on inclusion criteria but have been consistent in showing that adherence to a Mediterranean Diet reduces the relative risk of CVD by 30%.

Meta-analyses of observational studies have compared the highest Mediterranean diet adherence with the lowest category. One included RCTs and reported that following a Mediterranean diet resulted in a lower incidence of CVD (RR 0.76, 95%CI 0.68 to 0.83) and CVD mortality (RR 0.76, 95%CI 0.68 to 0.83) (Grosso et al. 2015). Another did not include RCTs and reported a reduced risk for acute myocardial infarction (RR 0.70, 95%CI 0.62 to 0.80,  $I^2=44\%$ ) and unspecified CVD (RR 0.81, 95%CI 0.74 to 0.88,  $I^2=80\%$ ) (Rosato et al. 2017).

A cumulative meta-analysis of prospective studies (cohort and RCTs), sorted by year of publication, standardised adherence using Trichopoulou's 10-item score for an increase of two points in adherence to the Mediterranean Diet Score. They reported 11% reduction in risk of mortality from or incidence of CVD (RR 0.89, 95%CI 0.86 to 0.91,  $I^2=76\%$ ,  $n=27$  studies) (Martínez-González et al. 2017). One meta-analysis solely of RCTs with cardiovascular outcomes has also been attempted (Liyanage et al. 2016) but was of little value as it included inappropriate and disreputable studies. The existing Cochrane review also sought data from RCTs, but in the absence of studies with clinical events, reported a reduction in LDL-cholesterol of -0.07mmol/l (95%CI -0.13 to -0.01) from 6 RCTs (Rees et al. 2012). The dietary intervention only had to meet two of nine Mediterranean characteristics for the study to be included,

therefore the review was criticised for lacking specificity (Martínez-González et al. 2017). The inclusion criteria for the updated Cochrane review have been revised based on evidence suggesting the most likely active components of the Mediterranean diet. These components were identified during pooled risk analyses of individual elements of the diet showing a significant reduction of CVD risk for adequate olive oil consumption, vegetable intake, fruit, and legumes, with an increased risk for dairy products (Grosso et al. 2015). However, it was unclear what was considered 'adequate' and how the pooled analysis was conducted as this was not described in the methods, even as a subgroup analysis. The differences across versions of Mediterranean diet scores also present a major limitation, for example where nuts were included with fruit and legumes in five of the ten studies, rather than as a separate entity (Grosso et al. 2015). Thus, the new Cochrane review criteria state: 'Both of the following key components were required to reach our definition of a Mediterranean-style diet: high monounsaturated/saturated fat ratio (use of olive oil as main cooking ingredient and/or consumption of other traditional foods high in monounsaturated fats such as tree nuts) and a high intake of plant-based foods, including fruits, vegetables, and legumes. Additional components included: low consumption of meat and meat products and increased consumption of fish, moderate consumption of milk and dairy products.' (Rees, pers comm).

Currently, the key RCT remains the PREDIMED trial (1.3% weighting of total evidence in cumulative meta-analysis) from Spain where 7,447 adults with high cardiovascular risk were randomised to one of three groups: low-fat control, Mediterranean diet with a supply of virgin olive oil, or supply of nuts. The

Mediterranean diet reduced the incidence of major CVE by 30% (HR 0.70, 95%CI 0.55 to 0.89), translating to an absolute risk reduction of 3 major cardiovascular events per 1,000-person years (Estruch et al. 2018). These findings must be viewed cautiously for several reasons. Firstly at face value, the effect size presents uncertainty due to imprecision with the lower confidence interval crossing the minimal important difference of 0.75. Secondly, whilst it was pre-specified as the primary endpoint, the incidence of CVE was a composite of myocardial infarction, stroke, and death from cardiovascular causes. Due to low event rates, use of composite endpoints have become common in cardiovascular trials. However, the benefits of increased statistical power and avoidance of type II error need to be weighed against challenges with interpretation (Weintraub 2016). PREDIMED used time-to-first-event which assumes that all events are equally weighted and therefore of equal clinical relevance. Whilst the endpoints occurred at similar frequency, they would not be considered of similar importance to patients (McCoy 2018). It is therefore important to note that of the component endpoints, only the risk of stroke had a statistically significant reduction in the Mediterranean diet groups. As pointed out by the research team, a much larger sample size and longer follow-up would be needed to analyse total mortality (Guasch-Ferre et al. 2017). Thirdly and more importantly, the original PREDIMED study published in 2013 was withdrawn following suspected irregularities in the randomisation procedures highlighted by an independent analysis revealing that the distribution of baseline variables were significantly different from what would have been expected to result from randomisation (Carlisle 2017). The PREDIMED authors identified several departures from the randomisation procedures including: inconsistent use of the randomisation tables at two sites, an error in the

randomisation table resulting in fewer women allocated to the Mediterranean nuts group, assignment of intervention via cluster rather than individual at one site, and 425 participants who were not randomised but given the same intervention as their previously enrolled household member. Subsequent sensitivity analyses explored the impact of these, showing little effect on the effect estimates, and the study was republished in 2018 confirming the original findings (Estruch et al. 2018).

## **Diet for prevention of CVD in HIV**

### **Evidence from systematic reviews**

Within HIV guidelines, the guidance for management of modifiable risk factors to prevent CVD are based on evidence from the general population. Whether these findings can be extrapolated to the HIV population on ART is unknown. If dietary interventions were effective, CVD risk can be reduced through behaviour modification reducing toxicity and pill burden accompanying lipid-lowering medication.

Narrative reviews have examined the broader management of HIV dyslipidaemia including drug intervention (McGoldrick & Leen 2007) and the effect of nutritional support and exercise on body composition (Leyes et al. 2008). The only review to focus on dietary intervention was published in Portuguese and included 13 intervention trials and 7 observation studies. Citing issues with uncontrolled trials and small sample sizes, they concluded that there was little evidence for effectiveness of dietary interventions for HIV dyslipidaemia (Almeida et al. 2009). Given the lack of evidence, Stradling et al (2012) carried out a systematic review and meta-analysis of randomised controlled trials (RCTs) assessing the efficacy of dietary interventions or

supplementation for HIV dyslipidaemia. PRISMA guidelines for systematic reviews (Liberati et al. 2009) were followed, including protocol registration (PROSPERO 2011:CRD42011001329). A comprehensive search was conducted up to March 2012, as described in the paper (Stradling et al. 2012). Of the 18 RCTs that met inclusion criteria, 12 involved specific nutrient supplementation (omega-3, vitamin E, nicotinic acid, or chromium) and 6 involved dietary interventions (based on NCEP ATPIII dietary recommendations (NCEP ATPIII expert panel 2002)). Among the six diet studies, two studies were excluded from meta-analysis due to lack of data (Thanasilp 2009), and head-to-head design (Ng et al. 2011). Dietary intervention reduced triglyceride levels by  $-0.46$  mmol/l, (95%CI  $-0.85$  to  $-0.07$  mmol/l,  $P=0.02$ ,  $I^2=30\%$ , 4 RCTs,  $n=201$ ) compared to control over an average treatment period of 8 months in patients on ART. No significant differences were found between groups for total cholesterol ( $0.01$  mmol/l; 95%CI  $-0.71$  to  $0.73$ ,  $P=0.97$ ,  $I^2=85\%$ ), HDL-cholesterol ( $0.11$  mmol/l; 95%CI  $-0.01$  to  $0.22$ ,  $P=0.07$ ,  $I^2=33\%$ ), and LDL-cholesterol ( $-0.01$  mmol/l, 95%CI  $-0.81$  to  $0.79$  mmol/l,  $P=0.98$ ,  $I^2=87\%$ , 3 RCTs).

Limitations to this analysis included small number of studies, small sample sizes, and inclusion of participants with mild dyslipidaemia, hence restricting the capacity to detect changes in lipids due to Type 2 error. Reporting in studies was generally incomplete regarding study design and outcome data such that estimation of standard deviation was required in four studies. Despite the lack of evidence of statistical heterogeneity in the analyses, clinical heterogeneity was anticipated between dietary intervention studies due to sparse documented methodology, the complexity of the 284-page NCEP ATPIII diet guidelines (NCEP ATPIII expert panel

2002) and likely variations in their practical interpretation. In the absence of good quality evidence to support dietary intervention for CVD prevention in HIV, the protocol for the Best Foods For your heart trial (BFF) was written in 2011 and submitted to the National Institute of Health Research for consideration of funding. The proposed trial aimed to address previous limitations around insufficient duration and intensity of the intervention, focusing on modification of dietary patterns rather than nutrients.

### **Evidence from RCTs**

Since publication of Stradling et al.'s meta-analysis, 5 studies have been published. Two studies employed diet alone as the intervention (Table 2), whilst another three incorporated diet as part of a lifestyle intervention (Table 3). Most of the studies opted for a dietary intervention based on NCEP ATPIII guidelines, which produced a modest reduction in LDL-cholesterol (Almeida et al. 2011; Chotivichien, Arab, Prasithsirikul, Manosuthi, Sinawat & Detels 2016a; de Figueiredo et al. 2013). However, the reliability of these findings was marred by the high risk of bias due to inadequate reporting of sequence generation and allocation concealment, anomalies between reported numbers, and high attrition rates. The favourable increase of HDL-cholesterol observed in the Boston study was more likely to be due to the exercise component of the intervention (Fitch et al. 2012), although this was not evident from the other RCT endorsing a weekly exercise programme (Saumoy et al. 2016).

Table 2 Studies using dietary intervention alone for prevention of CVD in HIV

Study	Design, aim and duration	Sample and setting	Intervention	Control group / adherence method	MD between groups / within IG change	Power calculation / attrition
Almeida 2011	RCT to prevent metabolic abnormalities, FU 1 year	N=53 on ART <1year, LDL 2.76mmol/l, 80% male, mid Brazil	Every other month. Individualized counselling by RD, F&V, wholegrain, fish, low sat fat, trans and sugar, based on WHO	CG: no advice 24hr food recall	MD: NS IG: LDL-0.18mmol/l, p=0.047	No power calculation. LTFU IG = 2/25, CG=9/28.
Chotivichien 2016	RCT to reduce LDL in pts on ART, FU 6 months	N=72 on ART >3m, LDL>2.6mmol/l, no LLM, 42% male, Bangkok	Monthly. Individual counselling <25% calories fat, <200mg cholesterol, <7% sat fat, based on NCEPIII	CG: no advice 24hr food recall	MD: not reported IG: LDL -13%, -0.56±0.1mmol/l, p=0.009 48% adhered to diet	50% power to detect 1mmol/l change in LDL with 72. LTFU IG = 6/35, CG 7/37.

Key: FU follow-up, LDL LDL-cholesterol, LLM lipid lowering medication, RD dietitian, F&V fruit and vegetables, sat saturated, IG intervention group, CG control group, MD mean difference, NS non-significance, LTFU loss to follow-up



Table 3 Studies using lifestyle intervention for prevention of CVD in HIV

Study	Design, aim and duration	Sample and setting	Intervention	Control / adherence	MD between groups / within IG change	Power calculation / attrition
Fitch 2012	RCT to reduce CAC score, HDL, CRP in pts with metabolic syndrome, FU 1 year	N = 42 in 2x2 factorial design, stable on ART >6months, 76% male, Boston	Weekly. <35% calories fat, <7% sat fat, exercise 3 hours/week Based on NCEPIII IG2: 500mg metformin twice daily	CG: placebo 4-day food diary	MD: NS in CAC, cIMT, hsCRP, fibre, fat or saturated fat intake HDL+1.01mmol/l p=0.03	85% power to detect 1SD change from baseline in CAC score, cIMT, MS parameters, 10 in each of 4 groups. LTFU IG1 = 2/21 IG2 = 4/21
Saumoy 2016	RCT to reduce lipids, CVR and cIMT, FU 3 years	N = 54, VL<40, CVR >10%, 100% male, Spain	Every 4 months. Meal plan from RD to reduce red meat, sugar, increase fish, olive oil, fruit and vegetables; aerobic exercise 3xweek; motivational interviewing to quit smoking. Not referenced.	CG: leaflets on diet, exercise and smoking cessation. Dietary intake not assessed	MD: NS in lipids, CVR, biomarkers; cIMT 0.041mm/yr p=0.03 IG: TC -0.7mmol/l p=0.02 CG: TG -0.8mmol/l p=0.01	No power calculation; LTFU IG=3/27. CG=1/27 10 patients in each arm started LLM during study, 25 were already on LLM at baseline
Webel 2018	RCT to increase physical activity and diet quality, FU 3 months	N = 107, VL<400, CVR>20%, 65% male, Cleveland US	Weekly. Group session: ½ hour exercise + ½ hour behaviour change including diet. Based on DASH / Mediterranean diet	CG: single visit 24hour diet recall	MD: -0.9kg P=0.03, NS in Healthy Eating Index, time in physical activity IG: -0.7 ±3.7kg at month 3	90% power to detect difference(unspecified) in moderate to vigorous physical activity LTFU IG: 3/51 CG: 2/50

Key: as above for table 2, CRP C-reactive protein, CAC coronary artery calcium, cIMT coronary intima media thickness, DASH dietary approach to stop hypertension, HDL high-density lipoprotein cholesterol, MS metabolic syndrome, SD standard deviation, TC total cholesterol, TG triglycerides, VL viral load

In summary, of the 5 studies presented here, 3 recruited insufficient numbers to draw statistical and clinically significant conclusions. There is a need for well-designed trials to examine the effect of different dietary patterns on CVD risk factors in the context of HIV.

### **Mediterranean diet in HIV**

The Mediterranean Diet has been examined in HIV cohort studies. Observational studies examining relationships between the Mediterranean Diet and metabolic parameters have also been conducted in the HIV population but failed to find associations with LDL-cholesterol. In a Croatian cross sectional study of 117 individuals, lipid levels rose during the first year of antiretroviral therapy as expected, but were not influenced by adherence to the Mediterranean diet (mean difference between adherers/non-adherers in LDL-cholesterol -3.9%, 95%CI -6.6 to 1.5,  $P = 0.46$ ) (Turcinov et al. 2009). A cross sectional study of 227 HIV infected adults on antiretroviral treatment in Boston found increasing adherence to the Mediterranean diet associated positively with HDL-cholesterol (standardised  $\beta = 0.15$ ,  $P = 0.01$ ) and inversely with insulin resistance (as measured by homeostasis model assessment HOMA of insulin resistance, standardised  $\beta = -0.13$ ,  $P = 0.02$ ), but only in the subset of patients with fat redistribution ( $n = 133$ ), as classified by a committee using dual energy x-ray absorptiometry (DEXA), single slice computerised tomography (CT) of lumbar spine, anthropometrics, and photographs (Tsiodras et al. 2009). Without the use of concomitant controls, bias and confounding are introduced, limiting the certainty of findings of observational studies such as these.

Clinical trials within the HIV population have included one parallel, randomised trial comparing low-fat NCEP diet versus the same with additional Mediterranean components. The findings were unfavourable in both arms, with increases in triglyceride levels in the NCEP group and increases in cholesterol levels in the modified Mediterranean group. It was, however, a pilot study, and variance was not reported, making interpretation difficult (Ng et al. 2011).

Another trial lacking a power calculation randomised 54 men at increased risk of CVD to one of two groups. The intervention was not explicitly NCEP or Mediterranean but contained general components of both, in addition to aerobic exercise 3 times a week and motivational interviewing to support smoking cessation, whilst the control group were given leaflets on diet, exercise and smoking cessation. The authors reported that lifestyle intervention improved lipids at year 2 but not at year 3 but this was unclear due to reporting of P values alone without confidence intervals or a table of results. Carotid intima media thickness progression was not prevented, advancement of which was worse in the intervention group. There was no measure of adherence to the intervention as dietary intake was not assessed. Lack of any clear outcomes may be due in part to the wide use of statins by participants, which were also commenced at month 4 when LDL>3.4mmol/l (Saumoy et al. 2016).

The addition of exercise to a dietary intervention with components from the Mediterranean diet was also employed by an American study aiming to improve diet quality. No significant difference was detected between groups for time in physical activity or diet quality, but weight loss was greater in the intervention group (-0.9kg, P = 0.03) (Webel et al. 2018).

In summary, the three trials conducted in the HIV population using a loose definition of the Mediterranean diet have failed to produce consistent outcomes. This may have been due in part to broad heterogeneity with variation in the study aims (such as reduce progression of subclinical atherosclerosis, improve diet quality, or prevention of dyslipidaemia), and population (Spanish, North American, Hong Kong). The quality of studies was also low with high risk of bias. The Mediterranean diet may be associated with improvements in parameters of metabolic syndrome for people with HIV lipodystrophy, such as HDL-cholesterol and insulin resistance, and may have the potential to promote weight loss when used alongside exercise. As a dietary pattern it merits further investigation in this population.

### Summary of evidence

Evidence to date from primary prevention trials in the general population show reduction in risk of CVD with the Mediterranean diet and LDL-cholesterol lowering properties from Portfolio foods. Neither dietary patterns have been appropriately tested in the HIV population.

### Rationale for intervention choice

Given that the understanding of the pathogenesis of CVD in HIV is limited, it would be naïve to presume that interventions to reduce CVD in the general population will translate to similar reductions in MI incidence in HIV populations (Mallon 2013). It is therefore essential that the effectiveness of dietary interventions are tested in people living with HIV. Previously NCEP style dietary interventions have failed to produce

sufficient improvement in blood lipid levels suggesting that greater intensity and duration are required in the HIV population. Based on current evidence in the general population, the proposed intervention for this trial comprised of both Portfolio foods to enhance reductions in LDL-cholesterol levels, and the Mediterranean diet due to promising reduction in CVE observed in primary prevention with multifactorial mechanisms of action, including reductions in inflammation (Ahmad et al. 2018; Casas et al. 2016), insulin resistance (Ahmad et al. 2018), plaque vulnerability (Casas et al. 2014), improved endothelial function (Schwingshackl & Hoffmann 2014), cholesterol efflux capacity (Hernández et al. 2017), and changes in lipid metabolites (Toledo et al. 2017). Thus, it is posited that greater intensity may be achieved by applying a dietary pattern and specific intervention foods to reduce CVR via different mechanisms.

## **Thesis aims and objectives**

To achieve the overall aim of this project, namely to examine the role of dietary intervention for people with HIV infection at risk of heart disease, several approaches were taken. Process evaluation alongside a pilot RCT was required in order to fully answer all of the research questions posed. Various definitions exist in the medical literature for the term 'pilot study'. The NIHR (National Institute for Health Research) advice emphasises the importance of executing a dummy run, 'a pilot study is a version of the main study that is run in miniature to test whether the components of the main study can all work together' (NIHR 2015). Whereas the MRC framework states that this is not necessarily the case, as the pilot study 'should address the main uncertainties that have been identified with the development work' (Craig et al.

2008). The current study encompasses both definitions, as it is a pilot study within a feasibility study (Eldridge, Lancaster, et al. 2016).

- 1) To pilot the procedure and design of the RCT.
  - a. To obtain reliable estimates for recruitment, retention and study attendance compliance.
  - b. To determine estimates of the variability of potential future outcome measures.
- 2) To assess feasibility of future RCTs.
  - a. To explore appropriateness of trial procedures, design and duration.
  - b. To examine acceptability from participants' perspective.
  - c. To assess impact of intervention on quality of life, gut function and vitamin status.
- 3) To assess potential effectiveness of Mediterranean Portfolio dietary intervention on:
  - a. LDL-cholesterol powered to detect 0.5mmol/l or 10% difference between groups (proposed primary outcome),
  - b. Blood lipids and other CVR factors including arterial stiffness,
  - c. Anthropometric measures,
  - d. Immune function and inflammatory markers.
- 4) To assess adherence to dietary intervention during the trial.
  - a. To measure nutrient intake before and after intervention.
  - b. To assess adherence to Mediterranean and Portfolio dietary patterns.
- 5) To conduct process evaluation of the intervention.
  - a. To assess fidelity, dose, uptake and reach of intervention.
  - b. To investigate acceptability of the intervention.
  - c. To assess participant responses to intervention.
  - d. To identify contextual factors associated with variation.

## CHAPTER 2: METHODS

### Personal perspective

To put this study in context, it is necessary to first explain the background. Shortly after the introduction of highly active antiretroviral treatment regimens in the late 1990s, patients started reporting body shape changes and unforeseen metabolic side effects such as high blood levels of glucose and lipids. Questions and concerns from patients were frequently raised in our HIV clinic about elevated cholesterol levels and increased girth size. These discussions led to an active partnership between patients and clinicians with the development of a care pathway and HIV-specific Metabolic Clinic. As patients tried different dietary approaches, guided by the dietitian in the Metabolic Clinic, ideas for a clinical trial emerged. The trial design has evolved over a long period of time. In 2009 I was invited to present my research ideas at the Rank Prize International Symposium on Nutrition and HIV infection. It was a unique opportunity to debate methodological issues with the leading researchers in the HIV field, such as choosing a continuous variable (like LDL-cholesterol level) as the primary outcome to enable a smaller sample size, and the nature of the control group, opting for an intervention of 'usual care' to minimize bias at the risk of an inadequate differential between groups. Whilst deliberating the detail of trial design it became clear that a more structured process was required to evaluate an intervention as complex as diet. Guided by the Medical Research Council Framework for Complex Interventions (Craig et al. 2008), the protocol was then radically revised in 2013.

Complex interventions are defined as interventions with several interacting components (Craig et al. 2008). For dietary interventions the interacting components consist of a vast array of nutrients within foods, that are consumed by participants irrespective of whether they form part of the intended intervention or not. The complexity of the intervention also exists in several dimensions. Any major change in diet potentially requires changes in shopping, cooking and eating behaviour that can be challenging for the individual to implement within their social and economic circumstances. The intervention also requires a certain degree of tailoring to ensure that it is implementable in daily routine for each individual. Therefore, the dietary intervention for the intended RCT was systematically developed along the five phases proposed by the original Medical Research Council Framework for Complex Interventions (Campbell et al. 2000).

For the first 'pre-clinical' step I conducted a systematic review of randomised controlled trials that used dietary intervention for the treatment or prevention of HIV dyslipidaemia (Stradling 2012), as described in the previous chapter. The findings indicated a lack of well-designed, robust trials. The intervention used in these trials (National Cholesterol Education Program Diet) failed to produce sufficient improvement in blood lipid levels, suggesting that greater intensity and duration are required. Evidence from non-HIV populations supports the use of portfolio foods to enhance reductions in LDL-cholesterol levels and the Mediterranean diet for reduction in mortality.



## MRC Evaluation Framework for Complex Interventions

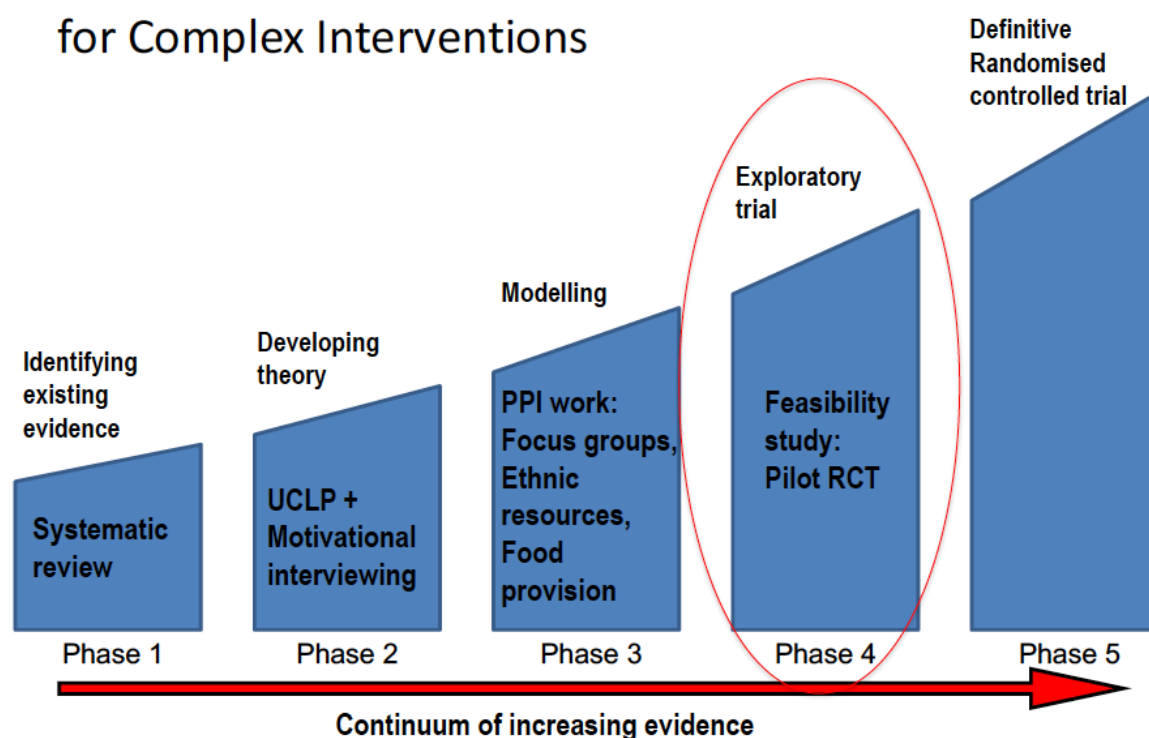


Figure 6. MRC evaluation framework for complex interventions

Source: Adapted from Medical Research Council (Campbell et al. 2000) page 3

Similarly, existing evidence suggests that using Motivational Interviewing as the method of delivery would be more likely to result in an effective intervention, due to application of the underpinning theoretical understanding of the process of behaviour change. Thus, the components of the complex intervention emerged from various sources (past practice, existing evidence, behaviour change theory) and are used in combination to enhance diet intensity.

The second step of the framework is the piloting and feasibility testing process. A focus group was convened with users of the HIV Metabolic Clinic to elicit their views both on research in general, and this specific dietary study. Group participants had been diagnosed with HIV infection for many years, encountered a multitude of side effects, and had taken part in previous research, mainly drug trials. They viewed research positively, with mainly altruistic reasons for participating, although they also cited the opportunity of being off pills or reducing pill burden as a motivating factor; identifying a key incentive for this study. Discussion around potential research topics, personal needs and hopes regarding food and nutrition related health were far ranging, including immune boosting and identifying interactions between food and antiretroviral therapy, but participants were unanimous in prioritising the key research question as 'how to reduce belly size and reverse lipodystrophy', hence the inclusion of waist circumference for consideration as an outcome measure. Patient involvement was also sought on an individual basis, from people who had already made lifestyle changes and achieved dramatic LDL-cholesterol level reductions, to seek their insight. Patients from the countries of origin represented by our HIV patient population were consulted on alternative options for meal plans and choice of foods to ensure acceptability and applicability across the entire target group.

Based on the evidence from this development process, an exploratory trial was proposed that would test the efficacy of the Mediterranean Portfolio diet in reducing LDL-cholesterol. An efficacy trial also presented the opportunity to pilot trial procedures such as recruitment, allocation, and retention rates to inform future effectiveness trials. Whilst the aim was to take an explanatory approach, in reality a

more pragmatic approach was required because this trial was being conducted within the 'real world' of NHS clinics under 'usual' rather than 'ideal' conditions. Clarification of trial design, to ensure appropriate applicability of findings, can be sought by using the PRECIS tool (Loudon et al. 2015). Ideally this is conducted during the design stage with discussion among the trial team. However, in this scenario it was used post-hoc, shown in Figure 7, to illustrate the degree of morphing that the protocol underwent during its design, towards increased pragmatism. The rationale for the scoring of each domain is given in Appendix .

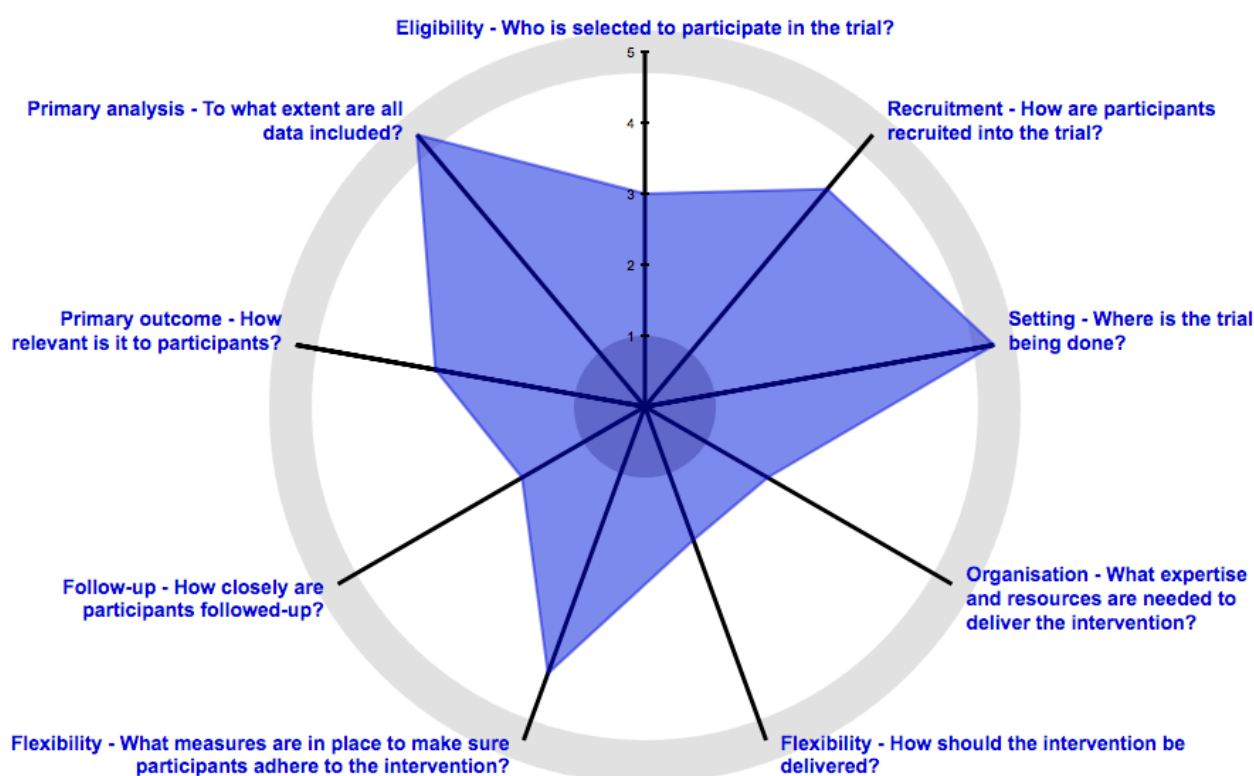


Figure 7. PRECIS diagram for the Best Foods For your heart trial

The structure and content of the trial protocol adhered to the SPIRIT guidelines (Chan et al. 2013) and was submitted to the ethics committee for consideration and approval. Prior to completion of the trial, the protocol was submitted for publication in BMJ Open, excerpts of which are presented here (Stradling et al. 2016).

## Published protocol: Methods and analysis

### Design

This is a two-arm, parallel, multicentre RCT for people with HIV dyslipidaemia. The two arms are:

Diet1 – low saturated fat ('usual care', see page 72)

Diet2 – Mediterranean Portfolio Diet.

Randomisation will be stratified according to gender and smoking status. The trial is funded by the National Institute for Health Research (NIHR) Doctoral Fellowship Programme and has been registered with the International Standard Randomised Controlled Trial Number registry (identifier: ISRCTN32090191). The study flowchart in Figure 10 shows progression through the study for individual participants.

### Setting

The study is a multicentre study set in 3 HIV services in the West Midlands (Birmingham Heartlands Hospital, Queen Elizabeth Hospital Birmingham, City of Coventry Health Centre). The West Midlands represents 10% of the population of England and Wales, and spans the range of population densities, urbanization, and socioeconomic profiling typical of the United Kingdom. It has the largest ethnic diversity outside of London with 17.3% of the population African, Caribbean or Asian (Wallace et al. 2014).

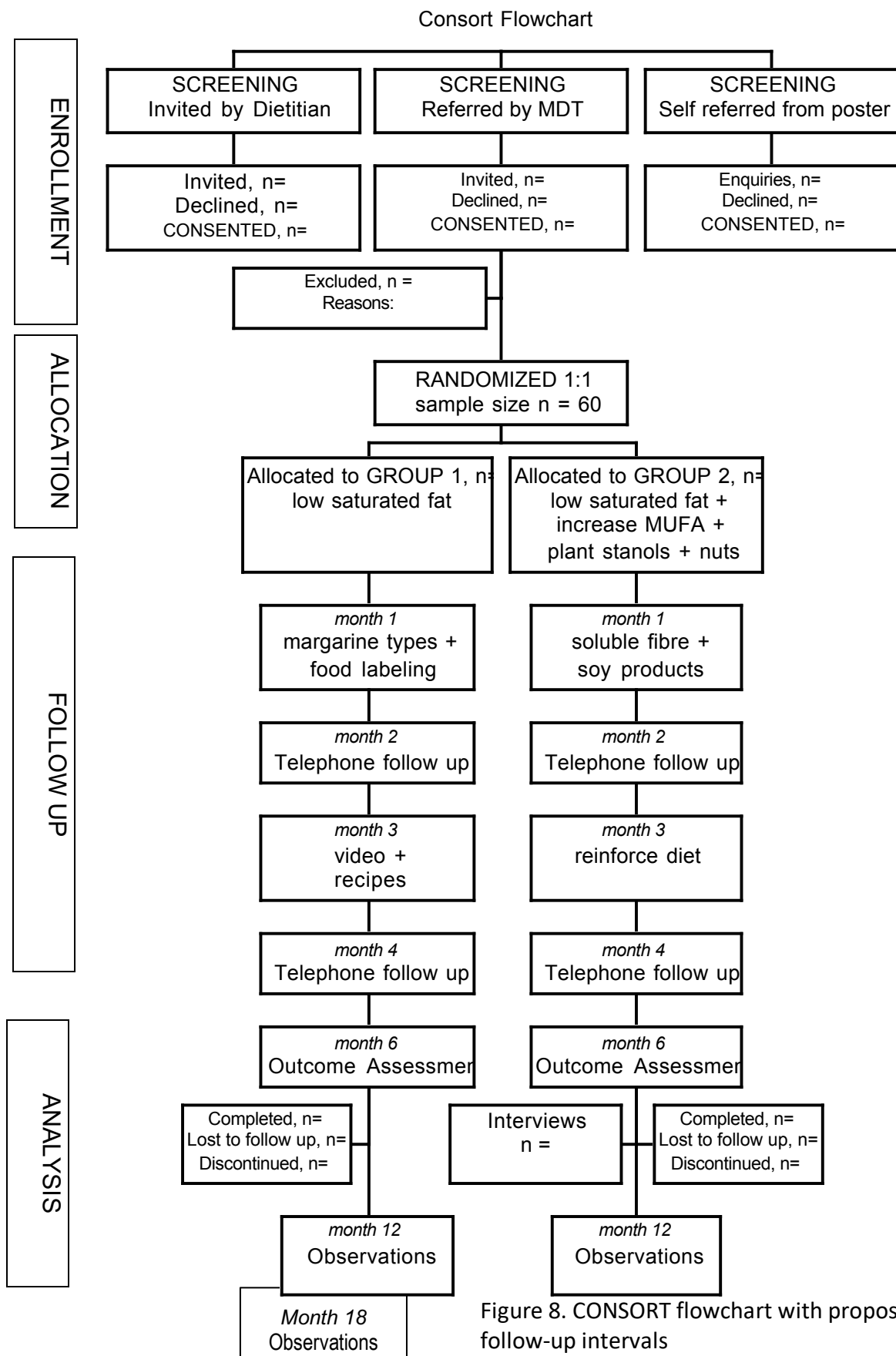


Figure 8. CONSORT flowchart with proposed follow-up intervals

## Participants

The target population is adults attending HIV services within the West Midlands, who have been stable on ART treatment for more than 6 months, viral suppression, and have LDL-cholesterol greater than 3mmol/l. The inclusion criteria are: fluent in English and planning to stay in the UK for 1 year. The exclusion criteria are: planning pregnancy in next 6 months; current use of lipid-lowering agents (any interfering drug or diet); secondary causes of dyslipidaemia (renal or liver disease, diabetes, hypothyroidism, familial hyperlipidaemia); known nut allergy; unstable psychiatric disorder (including eating disorders); current participation in a weight loss programme or other dietary intervention; inability to understand printed materials. When in doubt, the opinion and approval of the trial investigation team will be sought.

## Sample size

As this is a feasibility study a confidence interval approach was used to estimate the sample size required to establish feasibility (Thabane et al. 2010). The criterion for success is that a future effectiveness trial will be feasible if the attrition rate is less than 20% (or complete follow up in at least 80% of all recruited participants). With a sample size of 60 participants, the expected attrition rate of 20% can be estimated with a margin of error of 20% (95%CI 10 to 30%). This upper bound of 30% is appropriate as it is the cut off used to determine which trials constitute best evidence for behavioural intervention (Lyles et al. 2006) and qualify to receive funding for implementation by the Center for Disease Control and Prevention 2010 (Centers for Disease Control 2007). This sample size of 60 was also adequate for the proposed

primary outcome to determine a clinically relevant difference in LDL-cholesterol between groups in the RCT (see page 76).

## **Recruitment**

Recruitment will be conducted by a number of methods: advertising poster in waiting room for patients; referral from multidisciplinary team (MDT) providing HIV care; screening of patient blood results for LDL-cholesterol >3mmol/l. Patients will be assessed for eligibility by the researcher. Eligible patients will be handed or posted an invitation letter and participant information sheet containing details of all the interventions and assessments to be expected. To avoid contamination, the description of the dietary interventions will be general and applicable to both groups. Patients will be given at least 24 hours to consider the information before being contacted to confirm the date of their next routine bloods appointment, and reminded to attend in a fasted state, with no food consumed 12 hours prior to the appointment time. Written informed consent will be obtained from all participants at their first trial visit, prior to baseline measurements.

## **Allocation strategy**

Participants will be randomly allocated to Diet1 or Diet2 in a 1:1 ratio on their second trial visit. A statistician from the University of Birmingham will produce a computer-generated allocation sequence using random block sizes of 2 and 4, stratified by gender and smoking status. Block sizes will be concealed until completion of the trial. The research dietitian will allocate participants according to the diet number



concealed in the next sequentially numbered, opaque, sealed envelope, relevant for their gender and smoking status. As this is a complex intervention, it is not possible to blind the participants or the healthcare professionals. Use of the terms Diet1 and Diet2 will be used with the aim of achieving participant blinding to the exact content of the diet and type of foods used, to prevent internet searching of diet titles and potential contamination between groups. The success of the level of blinding achieved will be evaluated using questions on intervention preference and self-belief in success.

### **Data to be collected at baseline, month 6 and month 12**

Socio-demographic data on age, gender, ethnicity, and occupational status will be collected. Biomedical data will be collected on weight, height, body mass index (BMI), waist circumference, body composition, and stool frequency/bowel habit. Weight and body composition will be measured in light clothing, barefoot, on a Class 3 Tanita BC420SMA weighing scale (meets Medical Device Directive and Non-Automatic Weighing Instruments European regulations) (Tanita, Tokyo, Japan). Participants will be asked to abstain from food and drink for 4 hours prior to the assessment. Height will be measured to 0.1cm using stadiometers with the supported stretch stature method. Waist circumference will be measured horizontally at the top of the iliac crest with a flexible non-stretching Seca tape to 0.1cm. Bowel habit will be assessed via self-report to ascertain any disturbances that may require fibre and fluid intake modification.

Lifestyle data will be collected on smoking status, alcohol intake, and cardiovascular risk factors to enable calculation of QRISK 10 year cardiovascular risk (Hippisley-Cox et al. 2008). Blood pressure (BP) will be measured seated, with automated sphygmomanometer (Omron, Netherlands), taking the average of 3 consecutive readings. Intensity of physical activity will be measured objectively using the Actigraph GT3X+ accelerometer (Actigraph, Florida, United States); a tri-axial movement sensor that also records step counts. It will be worn over the right hip for 7 days, during waking hours, as this is the period to obtain consistent measures.(Hart et al. 2011) Mean counts per minute will be compared between groups to identify any potential influence of physical activity as a confounding factor. Best practices will be followed with regard to monitor use protocols, calibration and analysis of accelerometer data (Ward et al. 2005).

Aortic pulse wave velocity, as a measure of arterial stiffness, is an independent predictor of cardiovascular risk and mortality (Sutton-Tyrrell et al. 2005). Aortic pulse wave velocity will be measured from the right carotid and femoral arteries with the Vicorder system (Skidmore Medical, UK) (Hickson et al. 2009). Duplicate recordings will be made, and saved once a steady, consistent pattern of pulse waveforms is achieved and maintained for at least 10 cardiac cycles.

Blood samples will be collected and stored for later measurement of Apolipoprotein A and B, inflammatory markers (highly sensitive C-reactive protein) and insulin sensitivity (fasting insulin, adiponectin). Vitamin E levels will be measured to monitor any potential effect of plant stanols on absorption of fat-soluble vitamins.

## Dietary assessment

A number of different dietary assessment tools will be used to enable cross validation of methods and exploration of a variety of different strategies for selective implementation in future trials. Adherence to a Mediterranean Style diet will be assessed using a 14-item tool used in the PREDIMED study (Estruch et al. 2018; Estruch et al. 2013; Martínez-González et al. 2012). Fruit and vegetable intake will be assessed using a two-item questionnaire, previously used in studies with healthy adults from diverse ethnic backgrounds living in a low income neighbourhood (Steptoe et al. 2003) and patients with CVD (Jackson et al. 2005) and validated against plasma and urine biomarkers. A compliance score will assess consumption of the functional foods components of the Mediterranean Portfolio intervention at month 6 and 12 (Jenkins et al. 2006). Dietary intake will be assessed using a 3-day food diary, recorded either on paper or on the mobile phone app MyNetDiary (MyNetDiary Inc, New Jersey). Kitchen scales will be provided to assess food quantities. The researcher will review the diaries with participants to clarify brands, methods of food preparation, and recall of missing foods (Cantwell et al. 2006). Where weights of foods are unknown, they will be estimated using information from Ministry Agriculture Fisheries and Food Portion Sizes (Food Standards Agency 1994). Nutritional analysis will be conducted using DietPlan7 dietary analysis software (Forestfield Software Limited, Horsham). Metabolomic biomarkers will be used to independently assess dietary compliance, including trimethylamine-N-oxide and 1-methylhistidine for oily fish, urolithin A glucuronide for nut intake, equol for soy isoflavone, and linoleate, oleate and palmitate for olive oil intake. Urine sample from 24-hour collection at baseline and month 6 will be stored for later metabolic profiling

analysis by mass spectrometry using an analytical platform based on  $^1\text{H}$  NMR and MS at Imperial College London (Ismail et al. 2013). The quantified metabolite concentrations will be individually and collectively regressed against biometric data and against the global metabolic profiles.

### Process Evaluation

Process Evaluation questionnaires will be used at baseline and month 6. Various approaches were selected and combined in the questionnaires to incorporate the components recommended by the Medical Research Council guidelines (Craig et al. 2008). Fidelity and quality of implementation of the intervention will be assessed using open response questions to get a general overview of acceptability, as previously demonstrated in the WATCH IT evaluation (Bryant et al. 2011), and specific Likert ranked questions to examine participant perceptions of the strengths and weaknesses of programme, as used in the SHED-IT evaluation (Morgan et al. 2011). Assessment of diet intrusiveness, the participants' intention to make changes, and self-belief in success, will be used to support clarification of causal mechanisms. Contextual factors associated with variation in outcomes will be identified via a validated measure of self-efficacy for fat intake behaviour containing three task-specific domains of self-efficacy (negative mood, positive mood, and food availability), which have been shown to predict different types of fat reduction behaviours in low-income populations, with variations according to race (M.-W. Chang et al. 2008). In trials, such as this one, where blinding is not possible, the patient's preference for treatment can influence the outcome, especially where the participant is not simply a passive recipient of the intervention, but is required to

engage and make lifestyle changes. Therefore, intervention group preferences will be elicited before each randomisation, and used subsequently in the analysis of covariance to investigate the influence on LDL-cholesterol of participants' preferences at baseline (Moffett et al. 2005).

### Quality of Life

One generic (EQ-5D) and one HIV-specific (MOS-HIV 35-item instrument) will be used to assess the impact of the trial on the participant's quality of life. These tools are deemed the most appropriate adjunct and HIV-targeted measures for use in HIV clinical trials (Clayson et al. 2006). As responses to the EQ-5D thermometer tend to reflect physical more than mental health, the Warwick-Edinburgh Mental Well-being Scale (WEMWBS) will be used to measure mental well-being. WEMWBS is a 14-item scale covering subjective well-being and psychological functioning that has been validated in the UK adult population (Tennant et al. 2007). Permission has been granted to use these questionnaires in this study.

### Interviews

Qualitative data will be collected from a sample of participants at the end of the 6-month intervention period. Participants will be purposively selected from the Diet2 arm to include men and women of different ethnicities, with high and low levels of adherence, to reflect the diversity and breadth of experiences of a wide range of participants. Semi structured interviews will be conducted using open ended, non-directive questions. The aim will be to understand participants' experience of implementing dietary changes by enabling them to 'tell their story', what they did and

how, as well as identifying barriers to, and enablers of success in making and maintaining diet changes. It is anticipated that there will be variations in the effectiveness of the dietary intervention for different individuals in different sociocultural settings. Analysis of the interview data will serve to describe and potentially explain these differences and the factors involved. By exploring participant views on the intervention, study design and delivery, the interviews will identify problems at the feasibility stage to prevent them occurring at the full trial stage.

Written informed consent for digital recording of the 30 to 60-minute interview will be sought prior to booking a mutually agreed time and place, enabling the interview to be conducted either in participants' homes or a clinic room with privacy. Participants will be encouraged to invite their partner, family member or other cohabitant to join in the interview and give their perspective on how dietary changes are lived.

The sample size will be around 10 although the actual number recruited will be determined at the point at which the researcher is satisfied that a good understanding of the barriers and facilitators to making dietary changes has been achieved.

Interviews will be transcribed verbatim. Data analysis will be conducted following the Framework Method: familiarization, identifying a thematic framework, indexing, charting, mapping, and interpretation (Ritchie & Spencer 1994; Gale et al. 2013). Data analysis and management will be supported by NVivo10 software (QSR International). A subset of interviews will be independently coded to verify interpretation. Where possible, data triangulation with cohabitants will be used to enhance plausibility, trustworthiness and transferability of the data analysis process.

Reflective notes will be taken in a field note diary immediately after each interview and throughout data analysis to maintain reflexivity of the researcher.

## **Intervention design**

All participants, in both groups, will be invited to attend 3 individual consultations with the research dietitian and receive further telephone reinforcement and support during the 6-month intervention period. Appointment times will be offered between 8 am and 7 pm, enabling flexibility for participants who are working. This will be followed by a 6-month maintenance period, with routine clinic visits only. The same research dietitian, experienced in HIV nutritional care, will provide all consultations. Clinician blinding is not possible. Clinical equipoise exists as there is no evidence in the HIV population that one intervention is superior to the other (Cook & Sheets 2011). However, this is not the case for personal equipoise, as the research dietitian acknowledges a personal preconceived preference towards the Diet2 intervention. In an attempt to minimise this bias, she conducted all consultations to the best of her ability, maintained her duty of care, and kept a reflective diary during the trial.

### **Diet1: low saturated fat**

Consultations will focus on reduction of saturated fat to <10% of energy intake, in line with UK guidelines (Cooper A et al. 2007; Lundgren et al. 2008) and evidence on reducing the risk of cardiovascular events (Hooper et al. 2011). Resources will be provided, such as written information, recipes, and online videos, covering various topics including sources of saturated fat, food swaps, food labelling, cooking methods, cheese facts, and margarine types (detailed in Appendix 10).

On completion of the 12-month outcome measurements, participants in Group 1 will receive the dietary information from Diet2 (Mediterranean Portfolio). Should they choose to adopt the diet, they will be given the option of having their fasting measurements repeated at month 18, at their routine bloods appointment.

#### **Diet2: Mediterranean Portfolio**

In addition to the information provided to group one, participants allocated to Diet 2 will receive advice and support to adopt the Mediterranean Diet supplemented by additional functional foods with cholesterol-lowering properties. This will be embedded within a motivational interviewing style consultation to include assessing readiness to change, utilizing decisional balance, reflective listening and open-ended questions to identify needs, motivators and barriers to changing their diet, helping the participant to develop and verbalise arguments for change (desire, ability, reason need and commitment), and diminish resistance to it. The aim is to help empower and motivate the patient to make the changes identified during goal setting for their specific change plan. The dietitian will use the food diary to guide the participant towards the dietary changes most pertinent to their current intake whilst considering socio-economic factors and family dynamics. Whilst the aim is for every participant in Diet2 group to be consuming a Mediterranean diet (see Appendix 10), this is not prescriptive, as goals will be negotiated individually with each participant during their first session and reviewed at each visit. Thus in line with complex intervention thinking (Hawe et al. 2004), the intervention is not standardised by form, but by function, based on its principle to educate, empower and motivate participants to make changes towards the Mediterranean Portfolio dietary pattern.



On the randomisation visit, daily consumption of 57g tree nuts and 2g plant stanols will be encouraged in the form of 2 handfuls of unsalted mixed nuts (almonds, cashew nuts, peanuts, brazil nuts, hazelnut, pecan, walnut, pistachio, macadamia nuts) and 50ml cholesterol-lowering drink. At subsequent sessions participants will be encouraged to continue with the nuts and stanols, whilst also aiming to eat 15g/d soy protein as soya milk, yogurt or dessert, tofu and meat substitutes, and adopt a Mediterranean style diet, with more fish, vegetables, fruit, olive oil, tomato based sauce (i.e. onion or garlic fried in olive oil with passata or tinned tomatoes) and approximately 15-20g/d soluble fibre from oats, pearl barley, lentils, beans, and flaxseed. Supplies of the functional foods (nuts, soy protein, plant stanols, oats, pulses) will be given to participants to offset the additional cost of making dietary changes and inconvenience of searching for unusual items in supermarkets.

It is acknowledged that trials requiring longer-term dietary change face challenges with attrition and adherence to study requirements, therefore some flexibility with dietary requirements will be provided in order to facilitate positive experiences and encourage commitment to the long term (Crichton et al. 2012). If the participant is unable to consume the full amount of one component, for example due to taste, they will be encouraged to maximize the contribution of other components.

In keeping with maintaining the integrity of a complex intervention, this trial will aim to standardise the function and process of the intervention, not the components themselves (Hawe et al. 2004), thus allowing context level adaptation, for example use of ethnicity appropriate resources. A sample of consultations will be audio

recorded, for subsequent assessment by an HIV clinical psychologist, to monitor the quality of the motivational interviewing and fidelity of the intervention.

## Outcome measures

The primary outcomes for this study are feasibility and acceptability of trial procedures for recruitment, retention, data completion, and the intervention. The protocol will be considered viable for a future effectiveness RCT without modification if the following outcomes are met:

1. Recruitment rates of at least 50% of eligible patients;
2. Attrition rate of less than 20% by 6 months;
3. Compliance rate of 60% to trial.

If these outcomes are not met, the protocol will be modified in light of the study's findings before pursuing the proposed primary outcome of LDL-cholesterol.

Recruitment rate will be measured as proportion of eligible patients who are subsequently enrolled. Methods of recruitment will be compared: difference between recruitment rate from systematic methods (screening results) and ad hoc methods (posters, referral by multi-disciplinary team). Reasons will be sought for declined participation. Appropriateness of eligibility criteria and their practical application for future trials will be explored.

Attrition rate is defined as both discontinuation and loss to follow-up of participants at 6 months. Logistic regression will be conducted to assess whether completion is

associated with age, gender, or allocation preference. Reasons for discontinuation will be sought from participants. The target of 20% (with precision of 10%) is based on the mean attrition rate from 5 portfolio diet studies (13%), and systematic review evidence (18%) (Stradling et al. 2012), although attrition has ranged from 25 (Ng et al. 2011) to 42% (Balasubramanyam et al. 2011) in other HIV diet studies.

Acceptability of the intervention and group allocation will be explored by questionnaire and assessed by comparing attrition rates between the two groups.

Attendance to appointments, collection of supplied foods and completion of food diaries will be used as indicators of participation and study compliance.

### Statistical analysis plan

Descriptive findings will be explored regarding numbers recruited, completing, dropping out, and summary characteristics of each arm at baseline. Confidence interval estimation, not P value, will be used as the appropriate analysis for pilot studies (Lancaster et al. 2004). For all binary outcomes, we will therefore compute the number and proportion in each arm, and 95% confidence interval (CI).

Exploratory analysis of the treatment effect will be conducted, and specifically for the proposed primary outcome, difference in LDL-cholesterol between groups at month 6. Intention-to-treat analyses will be conducted with subjects as originally allocated at randomisation to avoid bias. Significance levels will be set at  $<0.05$  with 2-tailed tests. Effect size for the performance outcomes will be explored using analysis of covariance (ANCOVA) with adjustment for baseline value (Vickers & Altman 2001)

and we will report mean differences in outcomes between arms with 95% confidence intervals. Standard deviation of each outcome will be estimated, to inform the power calculation for a future trial.

Further exploratory analysis will be piloted to highlight any potential modifications required for the future trials. This will include integrating process and outcome data to maximise the interpretation of results (Oakley et al. 2006). For example, to address the question of the relationship between LDL-cholesterol and variation in adherence levels to the diet, on-treatment analysis will be used, in which results for participants who adhered to the diet will be compared with results from those allocated to the diet (standard intention-to-treat approach). To address the question regarding differences in responses to the intervention between subgroups of participants, regression analysis will be used in the definitive trial (as the power will be insufficient in the pilot study), with tests for interactions to identify participants most and least likely to benefit from the intervention.

Although this trial is not sufficiently powered to compare outcome between intervention arms, estimates of differences between arms will be compared. In the event that the effect of the intervention is large (in the region of 0.5mmol/l or 10% difference in LDL-cholesterol reduction between groups at 6 months) this study will have sufficient power and it will be important not to miss such large effect sizes if they exist.

## Ethics and dissemination

The trial has been reviewed by the West Midlands Ethics Committee and has been approved (reference: 13/WM/0225). The Trial Steering Committee will provide overall trial supervision. The main ethical consideration is to ensure that the risk of harm to participants is minimized and that they are fully informed of any risks. Lipid lowering agents are not permitted during the trial. Should an individual's LDL-cholesterol rise above  $>5\text{mmol/l}$ , this will be recorded as an adverse event and they will be withdrawn from the study with GP referral for appropriate medication.

The research dietitian will monitor the condition of the participants during the trial at study visits, requesting evaluation of potential harms by the participant's HIV physician during their routine clinic visits. All adverse events, whether considered trial related or not, will be documented in the participant's case report form, and reported to the trial steering committee and Sponsor.

Participants will be free to withdraw from the study at any time. If the participant withdraws from the intervention, they will be asked if they are happy for data to be collected at routine clinic appointments.

Electronic trial data will be entered into the encrypted and password protected trial computer, stored on the secure hospital server, and archived for five years after study completion. Paper copies will be held in the research office for purposes of potential data checking and shredded 1 year after study completion.

## Further aspects of methodology

Various aspects of the methodology were pondered, deliberated, disputed and contested between student and supervisors during drafts of the trial protocol. Debate regarding certain components of the rationale was considered inappropriate for the published protocol but merit further discussion here.

## Ethical Issues

A couple of issues arose around the process of informed consent. Good Clinical Practice Guidelines (1996) require that a subject voluntarily confirms his or her willingness to participate in a particular trial. As the researcher had been involved in providing care to this patient group at one of the recruitment sites for the last 20 years, it was felt appropriate that precautions were taken to prevent 'consent under duress' with the potential for patients wanting to please their health care professional. Therefore, firstly the researcher reviewed the patient information leaflet with the participant, ascertained comprehension and answered questions. Then the process of signing the consent form was performed with a research nurse who was completely independent, both of the research team and the patient's clinical care.

The other ethical issue raised around informed consent involved what is regarded as 'adequately informed' in the Declaration of Helsinki (2008), or 'after having been informed of all aspects of the trial that are relevant to the subject's decision to participate' section 1.28 (ICH Expert Working Group 2001). It is generally accepted

that this would usually include a detailed description of the intervention for both arms of the trial. However, in this trial this was not considered appropriate due to the risk of contamination between groups. Blinding of participants as to the content of the two diets was essential as it is basic human nature to believe that any 'new' intervention or an intervention with more components is more likely to be beneficial than anything perceived as 'usual care'. If detailed information about the content of the two diets had been supplied, participants could modify their behaviour and 'mix' elements from the two diets being compared, thus increasing the risk of type II error where an effective intervention is rejected due to an observed effect size that is statistically non-significant. No statistical techniques can be used to correct for contamination.

Therefore, a general description of the dietary intervention that was applicable to both groups was provided so as to blind participants to the hypothesis in terms of which intervention was being tested. This was not a waiver of consent, but simply an alteration of requirements to permit the description of the intervention to be general, not specific. Thus adherence was observed to the moral foundation of informed consent, which stems from the principle of respect for persons (McRae et al. 2011).

To illustrate, the patient information leaflet stated:

“You will be given advice on how to follow either Diet1 or Diet2. Both diets involve changing the type of fat that you use. You will need to change some of the foods you buy and eat in order to follow the diet. You will be given practical advice on shopping and cooking to help you make these changes. For the first 6 months you may be

supplied with some of the recommended foods that are considered to be expensive or difficult to find.” (shown in full in Appendix )

Thus, the participants were ‘adequately informed’, as is required by Helsinki 2008, about the intervention and what was required, without the need to talk about specifics such as a handful of nuts daily.

## Contamination

As discussed above, steps were taken to prevent contamination between groups from the outset of the trial, thus preventing potential reduction of the point estimate of the intervention’s effectiveness. The strength of randomized controlled trials lies in the random allocation of an individual to either the drug under investigation or the placebo, so that they remain in their distinct groups, taking only one form of tablet: active or control. Dietary intervention trials are at greater risk of contamination between groups, due to the treatment under investigation being readily available in the public domain, where an individual in one group is likely to consume foods from the diet of the other group. For this reason, usage of common names for the diets was avoided, such as low saturated fat, Portfolio diet, Mediterranean diet, and Ultimate Cholesterol Lowering Plan, to prevent participants from searching on the Internet. For example, the charity Heart UK promotes the Ultimate Cholesterol Lowering Plan on their website and produces diet booklets sponsored by the soya company Alpro.



In anticipation of the risk of contamination by dietitians in the clinics recruiting to the trial, training was undertaken on how to convey the information in the patient information sheet with the language of Diet1 and Diet2, rather than reference to the specific functional food items. Resources, such as the Heart UK flipchart, were removed from display in clinic rooms. This was alongside the routine staff training regarding eligibility criteria and how to promote the trial, giving out patient information sheets in clinic and referral to the researcher. Subsequent reinforcement was included in recruitment update emails and ad hoc in clinic on a one to one basis. Despite this, there were three identified cases of being unable to recruit due to the patient's prior knowledge of the intervention.

Site initiation included training the Comprehensive Local Research Network (CLRN) research officer for each site on the use of the patient information sheet and how to explain to patients what the dietary intervention might look like; changing the type of oil you use in cooking, rather than a specific meal plan or list of banned foods. Each site had a different system of recruitment and referral unique to suit their environment and requirements. The CLRN officers were excellent at screening and referring patients for recruitment, highlighting the necessity of being registered as an NIHR Clinical Research Network Portfolio study.

Some sources of contamination were not anticipated. For example, on hearing that the intervention diet contained nuts/soya/stanols, health care staff at one site obtained the Heart UK booklet and gave it to patients. This practice did not come to

light until the latter stages of recruitment when a doctor asked for more copies of the booklet. Gentle probing revealed his resistance towards any randomised controlled trials, as he did not like his patients being randomised to the control arm. Following further discussion, he relented and agreed that the patient could be offered recruitment into the trial. Subsequent investigation revealed ineligibility, as the patient's cholesterol level was too low, questioning why the patient had been recommended dietary intervention in the first instance. This highlights the need for extensive training and education of all staff at recruitment sites, as CLRN research officers reported that recruitment was often hampered by basic ignorance of research practice in medical staff. For large multi-centre trials, where each centre is recruiting small numbers, this presents a huge logistical issue.

## Recruitment issues

The recruitment strategy followed a format conducive to integration with the NIHR Clinical Research Network that operates in the United Kingdom. Physicians within teaching hospitals are familiar with the formalities of collaborating with CRN staff to screen and recruit patients to studies. Comments from external reviewers from the United States suggested the use of newspaper advertisements, highlighting the differences between health care systems and served as a reminder not to assume a working knowledge of the NHS system when writing for international publication. Trial registration on the UK Clinical Research Network (UKCRN) website facilitated email requests from patients in Kent and London to join the trial. Inclusion of patients from

further afield was inhibited by the need for additional site initiation of their respective treatment centre, which was not feasible due to the geographical limitations of the single researcher. The alternative of travelling to one of the three host research sites was offered to external patients but this also necessitated follow-up of care for one year at the host site.

### Eligibility criteria

Enrolment into the trial was discussed with the participant's physician to determine that no change in antiretroviral treatment was anticipated, and thus satisfy the eligibility criteria. Some patients made drug treatment changes and returned six months later to enrol in the study. For others, physicians knew that patients were in the trial and discussed whether it was appropriate to delay the medication change until the end of the trial.

Exclusion criteria included diabetes and hypothyroidism, as these endocrinal disturbances would influence changes in glucose and lipid levels. An external reviewer suggested retaining these individuals and examining them as a subgroup, if recruiting the required sample size proved problematic. This was not viable in the feasibility study due to sample size but will be considered for larger future trials.

Participants with other sources of inflammation (autoimmune diseases, chronic viral or bacterial infections) were excluded indirectly by targeting a population of HIV infected individuals who were 'stable'. The rationale was to limit confounding

influences from sources of immune activation and inflammation other than the direct effect of HIV itself (Duprez et al. 2012).

Transgender individuals were given equal opportunity to access the study, with randomisation stratified according to their biological (and genetic) sex prior to surgical intervention. Gender was the term used throughout the protocol to mean the person's biological sex rather than social construct, and in preference to the term 'sex' which can be misconstrued as sexual behaviour/orientation.

### **Challenges Specific to Dietary Intervention Trials**

Dietary intervention trials raise many issues due to the nature of the intervention being complex by definition and requiring behaviour change. As participants are required to change eating habits, the difficulty with negative findings is knowing whether the intervention has failed or compliance to it (Baldwin et al. 2008). This was addressed by using multiple dietary adherence measures in this trial, not relying solely on self-report methods, but also biomarkers.

Issues highlighted by other authors on the challenges of running longer-term dietary intervention trials include high rates of attrition, and difficulties maintaining participant compliance (Crichton et al. 2012). In an attempt to avoid early attrition, a 'run in' period was created between the recruitment visit (when patients gave consent and blood samples) and the randomisation visit, when they returned with completed food diaries and entered the intention to treat analysis. To maintain low levels of attrition at

later stages of the trial, flexibility with appointment times was sought to ensure minimal time commitment from participants by linking study visits with routine clinical care. Participant compliance was promoted via the provision of key portfolio food items throughout the first six months of the trial. Other measures used to enhance retention and compliance were: assessment of participant motivation to make dietary changes at the screening visit; maintenance of regular contact with all participants from both arms via the extra telephone follow up; provision of flexibility with dietary requirements, for example when patients disliked the taste of soya, they could compensate with other functional food components.

Choice of comparator is a challenge in dietary trials. A control arm was required in this study to ascertain that any change observed in outcomes was due to the intervention diet and not due to other factors, such as the participants receiving more attention from being in a study, or heightened awareness of eating habits due to keeping a food diary, as both groups experienced this. Participants need to be randomly assigned to either the intervention or control group to equalise pre-existing individual differences across experimental conditions, such as ethnicity, age, degree of immune suppression, physical activity levels, background dietary habits, or type of HIV medication. Using randomisation and a control group increases internal validity of the study, thus allowing inference that any observed group differences in blood lipid levels is due to the independent variable of dietary approach. Statistically the best option would be to have a control group of no intervention. This raised several issues: introduction of performance bias due to differential levels of attention

between groups, potential barrier to trial recruitment and participation, and considered unethical in the local context where at Heartlands HIV Service patients received dietary advice as usual clinical care. Therefore, an active control group was considered appropriate, in line with Declaration of Helsinki (2008) where the 'new intervention must be tested against those of the best current proven intervention'. This was deemed to be reduction of saturated fat to <10% of energy intake, in line with National Institute for Clinical Excellence (NICE) guidelines for the prevention of cardiovascular disease (Cooper A et al. 2007). By receiving usual care, the active control group did not receive substandard care, and were not disadvantaged by entering the study, thus protection against non-maleficence was achieved. Use of an active control group (Diet1 low saturated fat) minimised, but did not eradicate, performance bias as the intensity of Diet2 was greater due to motivational interviewing and provision of foods, despite participants receiving an equal number of follow up sessions with matched resources (recipes, videos, information sheets).

### **Intervention rationale**

The hypothesis of the current study was to combine the effect observed in the general population of the cardiovascular event reduction from the Mediterranean Diet, with the cholesterol reduction from the functional foods of the Portfolio Diet, to be delivered in the motivational interviewing style. Motivational interviewing was developed by Miller & Rollnick and subsequently ascribed to the transtheoretical model of change (Rollnick et al. 2010). The clinical method of motivational

interviewing is based on 4 key principles: expressing empathy, developing discrepancy, rolling with resistance, and enhancing self-confidence. As a 'conversation about change' it motivates and supports people to feel positive about the benefits of health enhancing behaviours (Abraham et al. 2009), and has been shown to be effective in the domain of diet and exercise (Martins & McNeil 2009). Systematic reviews in obesity management (Tuah et al. 2011), diabetes prevention (Baker et al. 2011), and CV risk reduction in high risk groups (Ebrahim et al. 2011), indicate that such robust behavioural change strategies are key to effective lifestyle change programmes. Guidelines for diabetes prevention (Paulweber et al. 2010) and obesity prevention/management (NICE 2006) recommend individual level intervention using established, well defined behaviour change techniques (goal setting, relapse prevention, self-monitoring, motivation interviewing, prompting self-talk, individual tailoring, time management. In this study, Motivational interviewing was used to facilitate the development of individualised approaches to support behaviour change in relation to diet, enhance participant self-efficacy and motivate them to change, achieve their goals and maintain behaviours.

Creating a name for the intervention 'Diet2' was problematic as it contained components from various sources, with terms and credibility that varied across different countries. The backbone consisted of the Mediterranean diet, with emphasis on fish, grains, fruit, vegetables and olive oil in meals cooked from unprocessed foods, but needed to be consumed in a non-Mediterranean setting. Foods from the Portfolio Diet with cholesterol-lowering properties were also

incorporated. These foods have similarly been adopted by HEART UK in their so-called Ultimate Cholesterol Lowering Plan, but without reference to the term 'Portfolio' or Mediterranean Diet. Functional foods are defined in the United States as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains." (Special Committee of the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences 1994)p109. Their use is recommended in American guidelines as additional options to enhance the effectiveness of cholesterol lowering diets (NCEP ATPIII expert panel 2001; A. H. Lichtenstein et al. 2006; Krauss et al. 2000; Howard & Kritchevsky 1997). The interpretation is slightly different in Europe, where the health claims are categorised more specifically. The European Commission have authorised risk reduction claims for plant stanols (European Food Safety Authority 2009), barley beta-glucan (2011), and soya protein (2013) as having been shown to reduce blood cholesterol, but not peanuts, which were declined in 2011 (EFSA Panel on Dietetic Products 2011). Having considered all of these labels, the term 'Mediterranean Portfolio' was coined for the Diet2 intervention.

## Smoking

In the clinical environment, dietary intervention for cardiovascular risk reduction would usually be accompanied by advice on physical activity and smoking cessation. For the purposes of this research, the dietary intervention was already considered a complex intervention, as it has multiple dietary components. Adding further



intervention components would make it very difficult to identify which aspect, if any, was influencing the effect size, particularly with regard to blood lipid profile.

Reduction of saturated fat intake is known to lower LDL-cholesterol, whilst smoking cessation and increased activity raises HDL-cholesterol, thus making interpretation difficult. Smoking status is a confounding variable due to the increased risk of cardiovascular disease in smokers and ex-smokers. Therefore, randomisation was stratified according to smoking status so as to control for the potential influence of smoking as a confounding variable. There was no plan to change the state of an individual's smoking status during the trial.

### **Physical activity**

Participants were not advised to increase physical activity levels. As activity levels are acknowledged as a confounding factor, they were measured at baseline and month six using accelerometers to check that levels were similar between groups and over the duration of the trial.

### **Sample size**

Originally the study was powered for LDL-cholesterol as the primary outcome, but this was changed to attrition rate as the statistician advised against including a power calculation in a feasibility study. The participant number of 60 was chosen for three reasons. Firstly, it was the number estimated using the confidence interval approach (Thabane et al. 2010) to establish feasibility of retention rates. Secondly, for a pilot

trial, 30 participants per arm is cited as a general rule of thumb to estimate a parameter (Browne 1995), which in this case will be the mean and standard deviation values from the control group for LDL-cholesterol, pulse wave velocity, waist circumference, and 10-year cardiovascular risk. These options are being explored to select the most appropriate primary outcome measure for future trials, and this parameter data will be used to perform a sample size calculation for the definitive trial. Thirdly, early calculations for the proposed primary outcome indicated that a sample size of 30 per arm provided 80% power to detect 0.5mmol/l (or 10%) difference in LDL-cholesterol between groups at 6 months at 5% significance assuming standard deviation of 0.6 (or 10%). Therefore, whilst the sample size for this feasibility study was not calculated based on a power calculation for a primary outcome of effectiveness, the number of participants were deemed adequate to detect a clinically relevant difference in LDL-cholesterol between groups. This calculation for the proposed primary outcome was conservative and had not allowed for the adjustment for baseline values in the analysis, which would increase the power. For this calculation, standard deviation (SD) was assumed as that observed in previous trials: Portfolio foods baseline value SD 10% (Jenkins et al. 2011), plant based diet SD 0.6 (Gardner 2005), dietary intervention in HIV population SD 0.6 (Lazzaretti et al. 2012). Interestingly, an audit of the UKCRN database found that the median sample size per arm for pilot/feasibility trials with continuous endpoints was 30 participants (Billingham et al. 2013).

## Process Evaluation

During the early patient and public involvement (PPI) work, concerns were raised about the commitment required by participants in research trials, including frequency of attendance, sufficient information about the study to make an informed decision, cost, inconvenience & impact on family. Therefore, these issues were addressed in the study design with: monthly attendance as the maximum frequency, resources to include sample meal plans and level of compliance required, provision of key foods, re-imburement for travel, provision of recipes and meal plans suitable for household. All participants recommended delivery of dietary advice in a variety of formats, including one-to-one discussion (as “take more in if have talked about it”), and written information or video on the website (as “something to refer back to”), but declined supermarket tours (as “draws attention to you”), group sessions and cooking demonstrations (as “worried about what other people will think”). They all highlighted the importance of support, and made suggestions ranging from telephone/text support, to delivery of ‘trial’ ready meals to go in the freezer.

During the trial design phase, immense energy, effort and emphasis was focused on the acquisition of validated tools to capture relevant outcomes and participant feedback. This was integrated with process evaluation, which in the absence of previous experience, was modelled on case studies and published examples, as detailed in the protocol. Subsequently in 2014 the Medical Research Council published an extensive and extremely useful guidance document on process

evaluation (Moore et al. 2014). It would have been informative for study design; however, my protocol had already been written and been given ethical approval. It was therefore inappropriate to make major changes at that stage in the research, but raised points for future studies, for example, the use of a logic model to depict the intervention. Logic models can help clarify causal assumptions, and the identification of core questions based on assumptions that have the most limited evidence base. Therefore, a logic model was developed retrospectively, see Figure 9. It illustrates how the programme was intended to work, with process and outcome components.

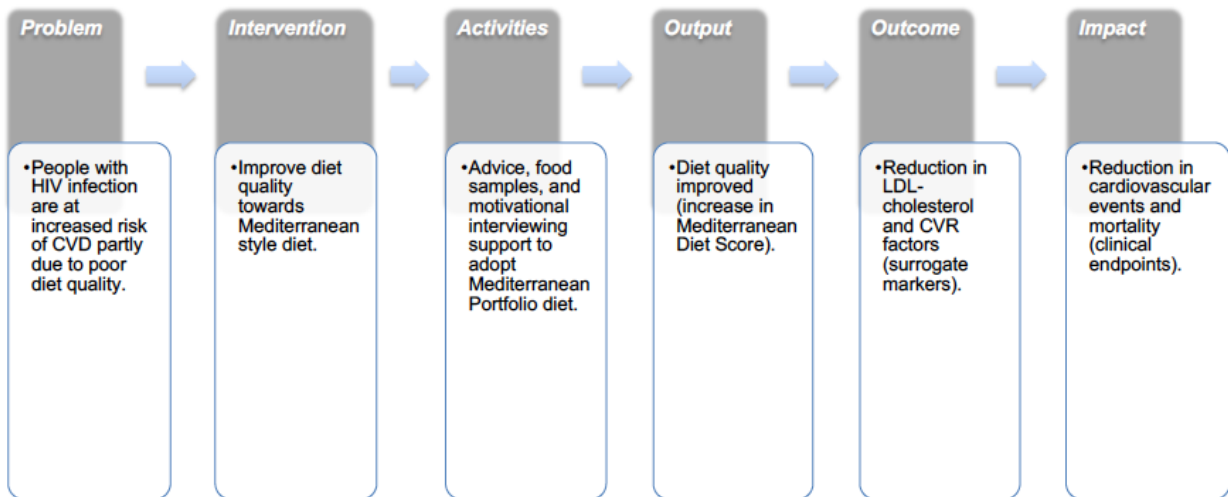


Figure 9. Logic model for Mediterranean Portfolio dietary intervention

## Protocol amendments

Two amendments to the protocol were required. The first was to permit changing the wording of the Mediterranean Diet Score (Appendix ). The original Mediterranean Diet Score was designed by Trichopoulou in 1995 when he examined traditional dietary patterns and survival in elderly Greek men (Trichopoulou, Kouris-Blazos, Wahlqvist, et al. 1995). The score was modified to adapt to other European populations, firstly in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, and then for the PREDIMED study (Martínez-González et al. 2012). This version was chosen because it had been validated (Schroder 2011), but it required clarification of certain terms to be understood within British culture, for example:

- ‘beans or lentils’ was inserted instead of ‘legumes’; ‘cakes, cookies, biscuits’ were added to ‘commercial pastries and sweets’ and were clarified as ‘not homemade’;
- ‘sofrito sauce’ was defined as ‘a tomato sauce (made with olive oil, onion, tomatoes)’;
- examples were given for ‘carbonated or sugar sweetened beverages’ – squash, fruit drink with added sugar, lemonade, cola.

Recruitment to the qualitative interviews proved difficult towards the end of data saturation, after about 10 participants. This was in part due to the size of the population being sampled, which was participants who had been allocated to Diet2 and completed 6-month follow up, of whom there were 27 individuals. It was also due

to the time commitment required and booking an appointment. Some participants vocalised difficulty in identifying a precise time in the future to book an appointment for the interview; therefore, agreeing to a telephone or Skype interview in the present moment was viewed as more preferable. An amendment was submitted to permit use of Internet video calls (using Voice over Internet Protocol applications such as Skype) or telephone to facilitate the qualitative interview. This adaptation of methods was required to recruit certain groups in the population to interview and facilitate a more diverse sample. The practical implications with regards to the protocol were that recorded verbal consent needed to be reaffirmed on the day of the interview, in addition to the usual written informed consent at a previous clinic appointment.

The issues raised in Ethics Guidelines for Internet-mediated Research (Hewson & Buchanan 2013) were largely irrelevant for this study, as they focused on blogs, forums and discussion groups where data is posted publicly online, and often anonymously, resulting in concerns over valid consent and the public/private distinction. In the current study, respect for the autonomy and dignity of participants was preserved with valid consent, confidentiality, and the option to withdraw.

Participants had the option to turn off the video conferencing and continue audio only and/or to stop the interview at any time. Thus, participating in the interview was entirely voluntary. Skype use standard internationally recognized and accepted encryption algorithms to protect communications from hacking and ensure privacy and integrity of data (Skype n.d.). Skype users have a digital credential to ensure

authentication, which is unnecessary for this study, as identification was confirmed visually, based on previous meetings face to face.

Other implications of using Voice over Internet Protocol applications that have been identified for qualitative researchers include rapport, disclosure, data quality, interaction and the research relationship. Rapport was unlikely to be an issue as the participant had already met the researcher on at least three occasions face to face during consultations and established a physical co-present encounter. Participants in the 'Your Space' survey described feeling comfortable with being interviewed remotely (Weller 2015). They also reported that remote modes offered greater privacy, convenience and flexibility, with informality that could be superior to physical co-present interviews, akin to communicating with friends or peers (Weller 2015).

## CHAPTER 3: FINDINGS

The main aim of this study was twofold: to assess the feasibility and acceptability of the Mediterranean Portfolio diet in people with HIV infection, whilst simultaneously piloting the design and delivery of a trial that will inform future evaluation of the effectiveness of dietary intervention on CVR of people with HIV dyslipidaemia. The specific objectives are detailed in Table 4.

The hypothesis was that the Mediterranean Portfolio dietary intervention would reduce LDL-cholesterol levels in people with HIV dyslipidaemia. A randomised controlled trial design was selected for this study, as it is the most rigorous method of determining whether a cause-effect relationship exists between an intervention and outcome. However, where the intervention is complex, effect sizes alone are insufficient to interpret the relevance of the findings to other contexts and understand how they might be applied elsewhere. The dietary intervention in this trial is described as a complex intervention because it has several interrelated components, some of which are tailored to the individual, and require participant behaviour change that may be perceived as difficult or challenging due to the level of skill required (Craig et al. 2008). The Medical Research Council (MRC) framework for complex interventions recommends that when using complex interventions, concurrent process evaluation is necessary within RCTs to explore the implementation, receipt and setting of an intervention and help in the interpretation of the outcome results (Oakley et al. 2006).



Process evaluation has three key functions: firstly to assess fidelity and quality of implementation, secondly to clarify causal mechanisms, and thirdly to identify contextual factors associated with variation in outcomes (Craig et al. 2008).

Therefore, the process evaluation for this study will be presented alongside the RCT findings in this chapter. As this study has been conducted in preparation for future trials, it is defined as a pilot study as a subset within a feasibility study (Eldridge, Lancaster, et al. 2016) and forms the 'feasibility and piloting phase' of the MRC complex interventions framework (see Figure 6).

The study consists of many overlapping parts: an RCT, a feasibility study, a pilot trial, mixed methods process evaluation, and a qualitative study using interviews. For this reason, whilst acknowledging the interrelated aspects and inevitable overlap, the findings will be presented within the following four sections to address the study objectives published in the protocol (Stradling et al. 2016):

1. Piloting the procedure and design of the RCT;
2. Potential clinical effectiveness of the intervention;
3. Process evaluation of the intervention;
4. Feasibility of the future trial.

Table 4. Summary of chapter structure

identifying study tools used to address study objectives and research questions

Research question	Objective from protocol	Tools used
<b>Section 1: Piloting the procedure and design of the RCT</b>		
Can a future RCT be done?	To obtain reliable estimates for recruitment, retention and study attendance compliance.	<p>Recruitment rate (proportion of eligible patients who were subsequently enrolled)</p> <p>Reasons for declining enrolment</p> <p>Interview Q1: reason for enrolment</p> <p>Attrition rate at month 6</p> <p>Appointment attendance</p> <p>Completion of food diaries</p>
<b>Section 2: Potential clinical effectiveness of the intervention</b>		
Should a future RCT be done?	To assess potential effectiveness using the surrogate outcome LDL-cholesterol, which was powered to detect a 0.5mmol/l or 10% mean difference between groups at month 6.	Baseline characteristics
Is it worthwhile?		Effect size estimated using multiple linear regression (ANCOVA) adjusted for baseline LDL-cholesterol
What sample size should be used for a future RCT?	To determine estimates of the variability of the outcomes measures and markers of CV risk, anthropometric measures and quality of life, including LDL-cholesterol, pulse wave velocity and waist circumference.	Standard deviation for each potential outcome measure to be used in future sample size power calculation

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### **Section 3: Process evaluation of the intervention**

#### *Implementation of intervention: fidelity, dose and reach*

To what extent was the intervention delivered as intended?	To conduct process evaluation to assess fidelity and quality of implementation.	Reflective diary Deviations from protocol
Dose & uptake – What was the uptake of the intervention?	To quantify levels of adherence to the dietary intervention.	Adherence levels
Reach – Who receives the intervention? Are they representative?	Does the intervention reach the intended target population?	Screening data

#### *Mechanisms of impact:*

What were the participant responses to the intervention?	To understand how the intervention works in practice.	PE2:2 Diet intrusiveness PE2:3 changes made Interviews Q2+5: response to intervention PE2:1 maintenance Likert
Unintended consequences?		Quality of life Vitamin E levels

#### *Context:*

Did external factors influence effectiveness?	To identify contextual factors associated with variation.	PE2:3 cooking skills PE1 intention to make changes PE1 readiness to change PE1 importance & confidence PE1 self and health belief PE1 self-efficacy for low fat foods
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#### ***Section 4: Feasibility of future trial***

How should a future RCT be performed?	To explore the appropriateness of the trial procedures, design and duration.	PE1 allocation preference PE2:1 allocation satisfaction PE2:1 types of support - acceptability
Is it feasible?	To examine acceptability from the participants' perspective.	PE2:3 strengths/weakness, suggestions for improvement Interviews Q6: changes to trial Food diary survey

Key:

PE1 Process evaluation questionnaire completed at baseline (Appendix )

PE2 Process evaluation questionnaire completed at month 6 (Appendix )

Numerical after colon denotes specific question number of tool that was used

## Piloting the procedure and design of the RCT

### *Section summary:*

This pilot study provides estimates that the current protocol would deliver recruitment of 35% of eligible patients to a future RCT, with >80% compliance to trial procedures, and <20% attrition. Extending the run-in period, widening inclusion criteria, and reducing time commitment required by participants could enhance procedure design further.

This first section of the chapter covers the pilot aspects of the study to answer the question; is a future RCT logistically possible in terms of design methodology and procedures?

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### **1. *Piloting the procedure and design of the RCT***

Can a future RCT be done?

To obtain reliable estimates for recruitment, retention and study attendance compliance.

The pilot study was powered to estimate the attrition rate with a 10% margin of error, requiring <20% attrition rate to establish feasibility. It was decided that the current pilot study protocol would be considered viable as the methodology for a future RCT without modification if the following conditions were satisfied:

- Recruitment rates of at least 50% of eligible patients;
- Attrition rate of <20% by 6 months;
- Compliance rate of 60% to the trial.

Each condition will be presented in turn.

## Recruitment rate of at least 50% of eligible patients

Study participants were recruited between October 2013 and August 2015 (over 23 months) from three different sites, the detail is shown in Appendix : Birmingham Heartlands Hospital (40 participants), City of Coventry Health Centre (13 participants, commenced June 2014), and Queen Elizabeth Hospital (7 participants, commenced October 2014). The recruitment targets for each site were 40, 10, and 10 respectively. The three recruitment centres were of equivalent size, each with a cohort of approximately 1,000 patients. Reasons for exclusion during screening are summarised in Figure 10.

Of the 882 patients assessed for eligibility at Birmingham Heartlands Hospital, 37% (326/882) had raised lipid levels ( $\text{LDL} > 3\text{mmol/l}$ ), 17% (146/882) were taking lipid lowering medication, 91% (801/882) were taking antiretroviral therapy, 85% (750/882) had an undetectable viral load ( $< 40$  copies/ml), and 16% were eligible for the study (145/882) meeting the inclusion criteria of: aged over 18 years, stable HIV infection without opportunistic infections, virally suppressed on ART for over 6 months, blood LDL-cholesterol level greater than  $3\text{mmol/l}$ , but not using lipid-lowering agents, absence of secondary causes of dyslipidaemia or known nut allergy, not currently in a dietary intervention programme, fluent in English and planning to stay in the UK for at least 1 year. Of the 145 subjects meeting the eligibility criteria, 34% were enrolled (50/145), and 28% were randomized (40/145). An equal number of men and women were enrolled. This represented a deliberate positive discrimination

towards women (when compared to 33% female present in the cohort), as women are usually under-represented in clinical trials. Proportions of the different ethnic origins were similar between those enrolled, declined, and screened, as seen in Table 5.

Participants attended trial visits from the time of randomisation (at baseline) for a period of 12 months for Diet2 (Mediterranean Portfolio) and 18 months for Diet1 (Low saturated fat). The trial follow-up period ended in February 2017.

In the protocol, the target recruitment rate was 50%, defined as the proportion of eligible patients who were subsequently enrolled. With this definition the recruitment rate was 42% (77/182). The target was not achieved for various reasons. Firstly, nearly half of those eligible declined 40% (73/182), the reasons for which were sought and will be discussed below. Secondly, the high number reported as 'no contact' (31 subjects, shown in Figure 10). This was defined as failure to attend clinic appointments, or cancellation at short notice, or when patients failed to answer their phone on seeing 'caller ID withheld' on their handset (all calls from hospital phones are registered as 'caller ID withheld'). Thirdly, it was not achieved because the target was unrealistic. The target was based on examples in the literature but was found to be overly optimistic due to the imprecise definitions of 'recruitment rate' used by studies. For example, a systematic review of recruitment and retention rates in 53 behavioural trials reported a mean recruitment rate of 49%, with 18 studies reporting

Table 5. Characteristics of patients assessed for eligibility from Birmingham Heartlands Hospital.

	Eligible (n=145)		Total screened (n=882)
	Enrolled (n=40)	Declined or DNA (n=105)	
<b>Male</b>	20 (50%)	76 (72%)	588 (67%)
<b>Female</b>	20 (50%)	29 (28%)	291 (33%)
<b>White European</b>	20 (50%)	49 (47%)	440 (50%)
<b>Black African/Caribbean</b>	17 (43%)	53 (50%)	391 (44%)
<b>Asian</b>	3 (7%)	3 (3%)	48 (5%)
<b>Mean LDL-cholesterol (mmol/l)</b>	3.9 ( $\pm$ 0.6)	3.9 ( $\pm$ 0.6)	3.0 ( $\pm$ 0.9)

recruitment rates >50% (Trivedi et al. 2013) providing evidence for the current selection of a 50% recruitment rate target. However, further investigation of the individual trials found that when recruitment was defined as eligible subjects who were enrolled, the actual recruitment rate was considerably lower (26% in (B.-H. Chang et al. 2004), and 35% in (Free et al. 2010)). Redefinition of the target recruitment rate as the proportion of subjects that progressed from enrolment to randomisation gave a value of 78% in the current study, this was consistent with other HIV trials involving dietary intervention, such as 86% in the 1-year Brazilian trial that aimed to prevent the development of dyslipidaemia on starting antiretroviral treatment (Lazzaretti et al. 2012).



As the target recruitment rate of at least 50% of eligible patients was not met, modifications will be required to the protocol for future trials. To further understand which modifications might be appropriate, reasons for both participation and declined participation were sought. These will be discussed later. Before that, we will examine another factor that affects the rate of recruitment; the eligibility criteria, as they determine the total number of eligible patients available within the cohort.

#### *Suitability of eligibility criteria*

The numbers of patients screened for eligibility, with their primary reason for exclusion are detailed in Figure 10 and Appendix . The exclusion criteria were found to be time consuming to apply due to the need to search through each medical history to check for comorbidities. The patient population frequently presented with numerous comorbidities and reasons for ineligibility; consequently, for ease of data presentation, the primary reason for exclusion was ranked according to the following hierarchy:

- i. On 3-hydroxy-3methylglutaryl-coenzyme (HMG-CoA) reductase inhibitors (statin medication);
- ii. Naïve to antiretroviral therapy or recently changed antiretroviral regimen in the last 6 months;
- iii. On antiretroviral medication but with detectable viral load (>40 copies/ml);
- iv. Had LDL-cholesterol <3mmol/l;

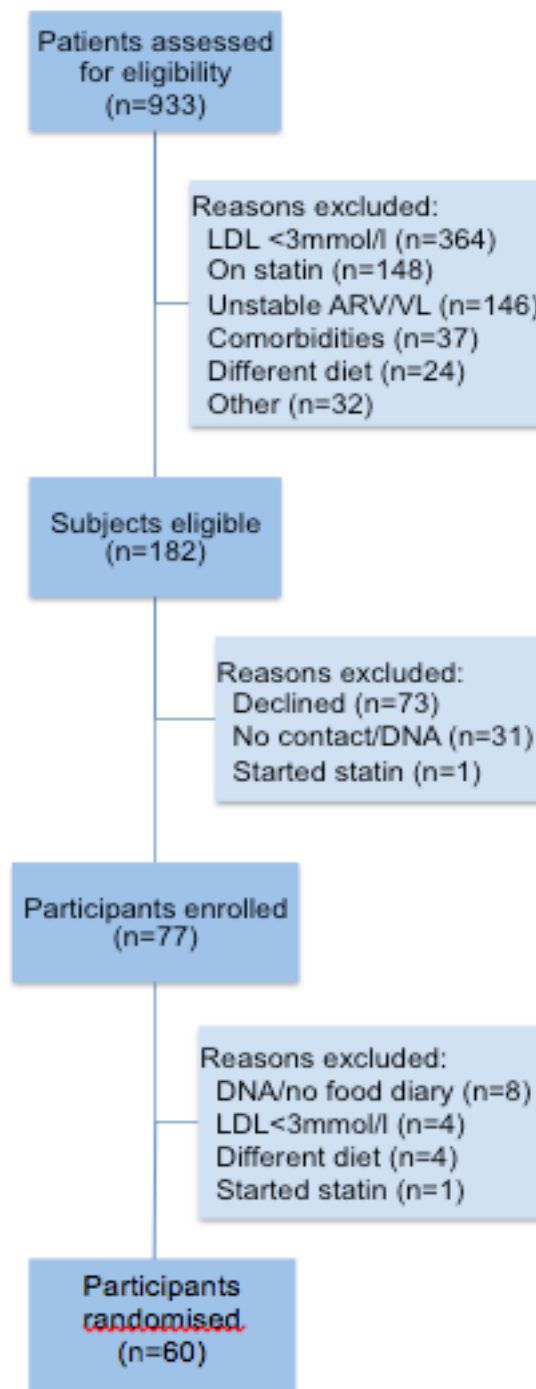


Figure 10. CONSORT Flow Diagram summarising participant screening and recruitment from Birmingham Heartlands Hospital.

- v. Already following a diet such as a weight reduction programme or not willing to make dietary changes;
- vi. Pregnant, in another research study, or in the military and therefore unable to commit to 1-year follow-up;
- vii. Language barrier;
- viii. Known psychiatric disorders including eating disorders, alcohol or drug dependency;
- ix. Co-morbidities that would impact blood lipid levels, such as renal disease, liver disease and cardiovascular disease.

For clarification, it is therefore important to note that the numbers presented in Figure 10 and Appendix do not represent the prevalence for each condition. For example, the table in Appendix indicates exclusion of 37 patients due to comorbidities, who had not already been excluded on the grounds of all the other criteria detailed in points i-viii above). Whereas examining the characteristics of all 737 who were ineligible, there were 15 patients with diabetes, 15 with kidney disease, 25 with liver disease, 3 with familial hyperlipidaemia, 9 with CVD, and 5 with hypothyroidism, giving a total comorbidity prevalence approximately twice that presented in the table.

#### *Reasons for declined participation*

Nearly half of the eligible patients declined entering the study. As shown in Appendix the most commonly cited reason was 'too busy', primarily due to studying or work commitments. More specifically, individuals explained that they were unable to get

time off work, had to book annual leave, or could not afford to have earnings docked for absence from work. Some people were juggling multiple jobs; their busyness was articulated by one man as,

*'Life is work, sleep, work, sleep, at the moment' (Black African male).*

Several people travelled over 100 miles to clinic and were therefore unable to manage the extra appointments at month 1 and 3 of the study, cited 'too far to travel' as their reason for declining. At the time when the study was designed, patients were routinely seen in clinic every 3 months for follow-up; however, this changed to 6-monthly during study recruitment. The reasons of time and distance, and others such as the financial burden of paying for parking or childcare whilst attending visits, are applicable to any research study, but other reasons were more specific to HIV studies. The stigma surrounding HIV remains strong, resulting in anxiety around the risk of disclosure of HIV diagnosis when individuals are seen in the 'HIV clinic'. For some, the idea of research was too frightening, as they felt that their confidentiality might be compromised by a change in routine,

*'I'm really not sure, you see I saw my best friend's husband there (MIDRU, the research building)' (Black African female).*

*'As a musician, I need my anonymity, so I only attend on Friday afternoons' (Black African female).*

A few were generally disinterested,

*'Don't need the hassle to be honest' (white male),*

*'Cholesterol will be okay on own I hope' (Black Caribbean female),*

Others voiced strong views about research,

*'Not interested in research, it won't help me; it feeds into the health industry, which is already large enough. I mean if people can't take care of themselves, don't need any more' (white male),*

*'F\*\*\* off, I ain't being no guinea pig' (white male).*

Complex social circumstances are common in this patient group (Rueda et al. 2016), and this was reflected by individuals feeling unable to commit to the duration or intensity of the trial due to life stressors. Gentle probing beyond the responses of 'too busy' or 'too stressed' revealed underlying reasons such as depression, anxiety, panic attacks, and recent bereavement. For others the 'can't commit' response was due to incarceration or indeterminate asylum status, awaiting indefinite leave to remain in the country.

One quarter of those who declined participation did so for reasons relating to the dietary aspect of the trial. Fasting overnight was required for accurate blood lipid results. This was a barrier for 5 individuals, who could not attend a morning appointment due to shift work patterns or feeling unwell when fasting. Seven individuals were not willing to change their dietary habits,

*'I am set in my ways regarding diet' (Black African female),*

*'Very picky about what I eat' (Black Caribbean female),*

*'I just eat what I want to' (White male),*

*'I'm a difficult eater, and am happy to do any research, but not with food' (White female).*

Another group of individuals (5) were concerned about the randomisation aspect of the trial, as they wanted a specific diet or to make changes on their own terms, such as undertaking exercise rather than changing their eating habits. The consent process operated smoothly and was not a barrier to recruitment. Hence no adjustment needs to be made to the consent process.

#### *Reasons for trial participation*

Efficient recruitment of adequately sized study populations is a well-known challenge to clinical trials. Therefore, in addition to exploring the barriers to trial participation, the motivating factors for trial participation were also sought. During the interview for the qualitative component of the trial, 16 participants in the Mediterranean Portfolio Diet2 arm were asked, "Why did you decide to get involved in the study?" Nearly all (14/16) described a personal interest in improving their health, with over half relating that to an increased awareness of their high cholesterol levels, and 3 wishing to avoid taking statins or further medication.

*'I think it was probably because I didn't really want to go on to more medication, more tablets. I did want to change my diet a bit just to make me generally more healthy' (White male).*

Themes revealed that it was this increased awareness of their cardiovascular risk combined with being asked or prompted by a member of the health care team,

*'If they [patient] know they're going to benefit out of it sometimes they listen more to their consultant because they say, "This is the man in charge of my life." (Black African female)*

A strong sense of altruism emerged in the themes of the interviews, with 7 describing an eagerness to help others. This element of philanthropy may be useful to guide the tone and content of future conversations during recruitment for clinical trials.

*'It was to do my bit, to give back a bit, I suppose' (White male)*

*'From my perspective it was something that I looked forward to in a way, because I thought, "Okay, I am taking part in a clinical trial, whether it finds that it has no effect or it has a positive effect, it is still of benefit to science and to the health of people, so we will see if it works for HIV positive people the same way it works in the general population." If it does great, we have proven that by adjusting your diet in small, and to be honest, manageable ways, you can lower your cholesterol. If it hadn't done that, we would have proven, "Okay, it works for the general population but obviously that means something else is happening in HIV positive people that it doesn't." So, there could be then further research from that. So whichever way it was going to benefit people in the future as to which way we try and deal with rising cholesterol levels in HIV positive patients.' (White male).*

### *Recruitment rate summary*

The target recruitment rate was not achieved; consequently, modifications will be required to the protocol for future trials. Findings suggest that the target recruitment rate needs to be lowered to around 10 participants per thousand patients as a more realistic goal.

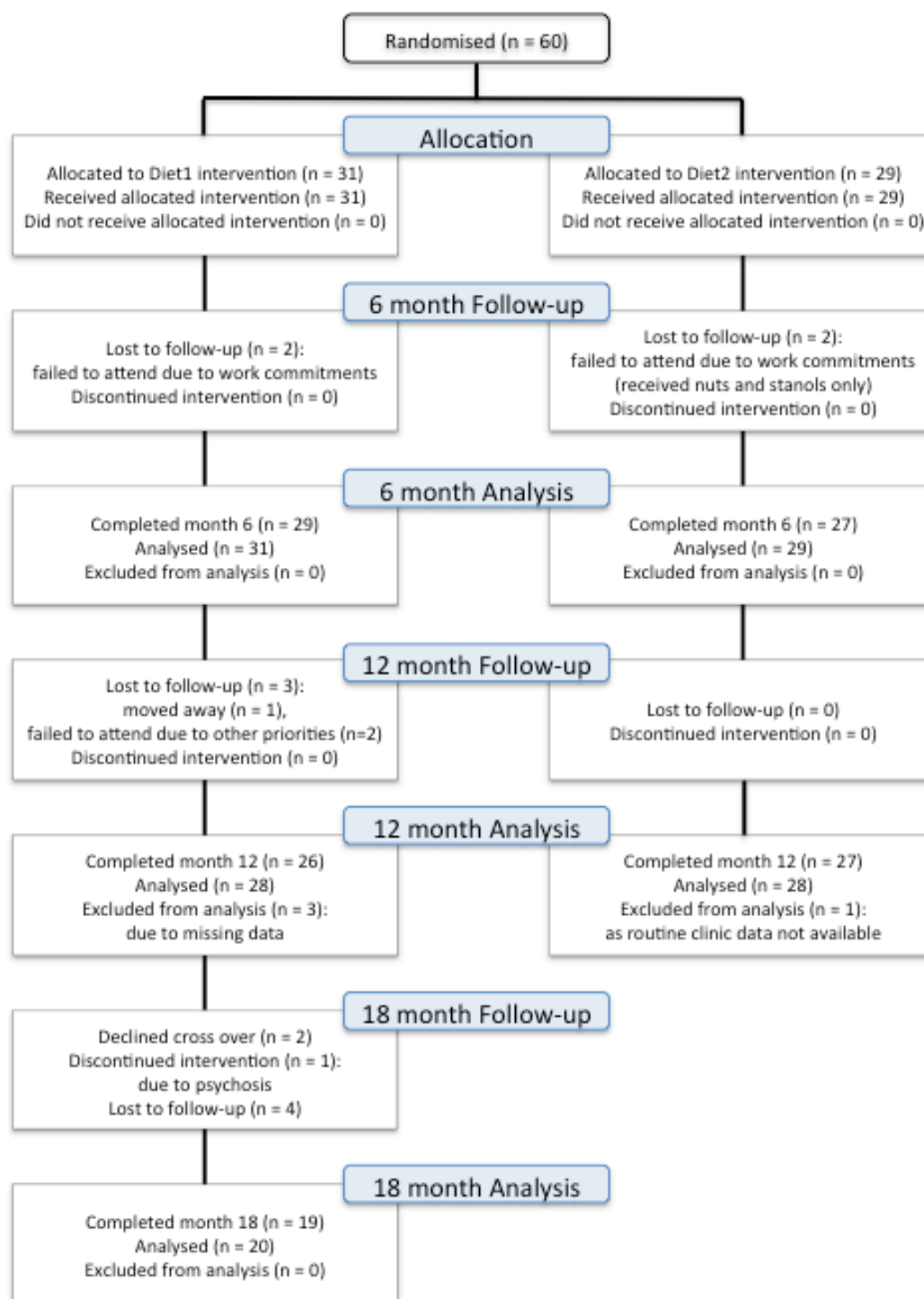
Widening the eligibility criteria and making the trial more attractive to patients could accelerate recruitment. Modifications could include refinement of the trial procedures with fewer outcome measurements and attendances to reduce the time commitment required, thus enabling patients to enrol who cited 'busyness' as their reason for declining. Eliminating the necessity for fasting would increase flexibility of appointment times. The researcher's use of a mobile phone would enable patients to identify the caller and prevent call barring. Exclusion criteria were found to be clinically appropriate but were time consuming to apply and elicited a small proportion of eligible candidates (16% of cohort). Comorbidities are common in this population, therefore removal of the list of comorbidities from the exclusion criteria would both increase the proportion of patients who are eligible and increase generalizability of the findings by improving external validity. There is no evidence that inclusion of people with comorbidities would compromise safety, as the Mediterranean Diet has been shown to be beneficial in those with diabetes (improving glycated haemoglobin, (Carter et al. 2014)), non-alcoholic fatty liver disease (associated with lower insulin resistance, (Baratta et al. 2017)), and chronic kidney disease (associated with lower mortality risk, (Huang et al. 2013)).



### Attrition rate of <20% by 6 months

The flow of participants through the trial from randomisation to month 18 follow-up is detailed in Figure 11. The frequency and reasons for dropping out before month 6 were identical between groups: time constraints to attend appointments due to work commitments, with two participants in each arm. Three of the four dropped out before month 1, such that those in the Diet2 arm failed to receive the complete allocated intervention, having only attended the session on nuts and plant stanols. After the 6-month follow-up visit, a further 3 participants were lost to follow-up from the Diet1 arm, due to moving away, and other priorities (social issues) inhibiting attendance to an appointment. Using exploratory analysis, no evidence was found of any differential attrition or clear predictors of drop out. Specifically, no evidence of association was found between completion of the trial and intervention group (OR 1.1, 95%CI 0.1 to 8.2,  $P = 0.9$ ), age (OR 1.0 95%CI 0.9 to 1.2,  $P = 0.7$ ), gender (OR 0.3 95%CI 0.03 to 3.4,  $P = 0.3$ ), or allocation preference (OR 0.4 95%CI 0.1 to 1.5,  $P = 0.2$ ). However, the wide confidence intervals indicate imprecision, suggesting that the sample size was too small for accurate estimations. As participants who dropped out of the trial were predominantly female (6/7), and of black African ethnicity (6/7), the level of commitment required by research participation may need deeper discussion with this group for future trials.

In summary, the total attrition rate was 7% (4/60 participants, 95%CI 3 to 16%) at month 6, and 12% (7/60 participants, 95%CI 6 to 22%) at month 12. Although greater



N.B. (n) refers to data for primary outcome, LDL-cholesterol

Figure 11. CONSORT Flow Diagram summarising trial allocation and follow-up.

than the 5% deemed to lead to little bias (Dettori 2011), this 7% attrition differed significantly ( $P = 0.01$ ) from the estimate of 20% factored into the sample size calculation to prevent any potential loss of power due to loss of follow-up. For that reason, loss to follow-up was not perceived to be a barrier to successful execution of a future RCT. Whilst 7% attrition is an acceptable level, it is acknowledged that the rate could increase when a future trial is scaled up with a larger sample size and more recruitment sites. Therefore, steps to improve the attrition rate could be taken, including reduction of participant time burden by reducing the number of outcomes measured during visits. One of the factors contributing to the low attrition rate in the pilot was the requirement for the baseline food diary to be completed prior to randomization. This could be extended by including a run-in period on the low saturated fat diet (common to both groups) in an attempt to prevent early attrition, as nearly half of the attrition (3/7) occurred at the beginning of the study, before the first follow-up visit at month 1.

### **Study Compliance rate of 60% to the trial**

Study compliance was assessed by two different measures: appointment attendance and completion of food diaries.

Participant attendance at research appointments was selected as an indicator of compliance within the study, as it reflected engagement with the process. Percentage attendance was calculated from the attendance of each participant at baseline,

month 1, 3, 6, and 12 study visits. Two telephone reviews (at month 2 and 4) were counted as an equivalent to the month 3 visit where it was missing (this applied to 4 participants). Low Saturated Fat Diet1 group attended 88% of their appointments (95%CI 81 to 95%), and Mediterranean Portfolio Diet2 attended 94% (95%CI 86 to 102%). The median attendance for both groups was 100%.

Food diaries were requested at baseline, month 6 and month 12. Completion of the baseline 3-day food diary was compulsory at enrolment before patients were permitted to proceed onto allocation. Completion of food diaries at month 6 was selected as a marker of compliance with the study process. Of the 29 participants remaining in the Diet1 low saturated fat arm at month 6, 26 completed food diaries (90%, 95%CI 74 to 96%), and 22 of the 27 in the Mediterranean Portfolio Diet2 arm (81%, 95%CI 63 to 92%). Thus, at month 6 food diaries were completed by 80% of the 60 participants (95%CI 68 to 88%). This was significantly higher than the 60% target for compliance ( $P = 0.002$ ).

In the protocol, collection of supplied foods was planned as an indicator of trial participation, but in practice, this was found to mirror appointment attendance and was therefore not considered an independent measure. Receiving study foods was also considered a key component of the intervention, such that when collection by the participant proved difficult due to transportation or storage, supermarket deliveries were arranged. This involved a significant time commitment on behalf of the researcher, with numerous emails and phone calls to confirm delivery times and

taste preferences. Therefore, it did not truly reflect the individual's engagement or participation in the trial.

Overall compliance rate to the trial processes was greater than 60% (91% appointment attendance and 80% food diary completion), therefore the methods were considered viable for future effectiveness trials.

### **Conclusion: Can a future trial be done?**

The pilot study completed recruitment to the target sample size of 60 participants. However, a 23-month duration, utilizing 3 recruitment centres was required to achieve this. Overall the study was a success as participants engaged with the intervention and completed outcome measures. Feasibility was established for future trials, as the attrition rate during the pilot study was less than the pre-specified 20%. Viability for a future effectiveness trial was established in part, as the condition of greater than 60% compliance rate to the trial processes was satisfied. The other condition regarding recruitment rate of at least 50% of eligible patients was not satisfied. Therefore, minor protocol modifications will be required to improve the speed and ease of recruitment. These include setting a more realistic target recruitment rate, widening the eligibility criteria, and reducing time burden on participants.

Overall, this pilot study has shown that a future definitive trial is viable and would be logistically possible in terms of design methodology and procedures.

## Potential clinical effectiveness of the intervention

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### ***Section 2: Potential clinical effectiveness of the intervention***

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Should a future RCT be done?	To assess potential effectiveness using the surrogate outcome LDL-cholesterol, which was powered to detect a 0.5mmol/l or 10% mean difference between groups at month 6.	Baseline characteristics, Effect size estimated using multiple linear regression (ANCOVA) adjusted for baseline LDL-cholesterol.
Is it worthwhile?		

What sample size should be used for a future RCT?	To determine estimates of the variability of the outcome measures and markers of CV risk, anthropometric measures and quality of life, including LDL-cholesterol, pulse wave velocity and waist circumference.	Standard deviation for each potential outcome measure to be used in future sample size power calculation.
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### **Should a future RCT be done and is it worthwhile?**

Examining the potential effectiveness of the intervention was considered an important objective in this pilot study to help secure future funding for future trials. The CONSORT extension for pilot trials acknowledges that pilot trials sometimes assess potential effectiveness using surrogate outcomes in a manner similar to phase II drug trials that assess the outcome to be used in a future phase III trial

(Eldridge, Chan, et al. 2016). LDL-cholesterol qualifies as both a surrogate measure for CVD and potential outcome to be used in a future effectiveness trial. Thus, the pilot study was powered to detect a 0.5mmol/l or 10% mean difference in LDL-cholesterol between groups at month 6.

In this section, the baseline demographic and clinical characteristics for each dietary intervention group will be presented to aid interpretation of the results and consideration of generalizability. This will be followed by an explanation of the rationale for statistical analysis plan selected and findings from each of the outcomes evaluated.

*Section summary:*

There was a significant difference between groups at month 6, after adjustment for baseline, with improvements in diet quality (Mediterranean Diet Score), intake of cholesterol-lowering foods, total cholesterol, total to HDL-cholesterol ratio, LDL-cholesterol, Apolipoprotein B, and systolic blood pressure.

This provides proof of concept that dietary advice to adopt Mediterranean Portfolio diet produces a clinically relevant reduction in cardiovascular risk factors in adults with HIV dyslipidaemia.

The sample size of 60 was adequate for a primary outcome of LDL-cholesterol or non-HDL cholesterol, but a 10 to 50-fold increase would be required to detect a difference in waist circumference due to the wide variation between individuals.

## Baseline characteristics

Of the 60 participants who were randomised, 52% were women, 65% were non-smokers, and average age was 42 years. A variety of ethnicities were represented, including black African (50%), and white European (40%). Country of origin was diverse and is represented visually by the word cloud in Figure 12. The majority of participants were first generation migrants having arrived in the UK in the last 10 years. Those of Asian ethnicity were mainly second generation.

LDL-cholesterol was mildly raised (3.9mmol/l) and participants had a low 10-year risk of CVD (QRISK 3%) despite a mean duration of antiretroviral treatment of 8 years. The majority of participants were overweight, mean body mass index (BMI) was 28 (SD 6), and 25% were obese.

Baseline characteristics of the dietary intervention groups were comparable for age, gender, ethnicity, smoking status, and lipid profile (see Table 6). There were no large systematic differences between groups. As expected with a small sample size there was some natural variation, such as longer duration of diagnosis with HIV infection in Mediterranean Portfolio Diet2 group. Any potential confounding would produce bias towards the null hypothesis and therefore would not inflate the effect size. P values were not reported in the baseline characteristics table for several reasons. Firstly, they are not an appropriate method of testing whether the randomisation was performed correctly, the methods section should inform this, as any baseline differences between the groups are by definition due to chance (Knol et al. 2012).



The randomisation process is expected to balance assignment between the groups, independent to the participant or investigator, and as such avoids systematic error (Festic et al. 2016). Any remaining imbalance will be by chance. Secondly, real, important, or meaningful differences between groups are often not detected in a statistically significant manner because trials are not powered for this purpose (de Boer et al. 2015). For example, the size of the difference in socio-economic status between groups can be observed in the numbers in Table 4, P values offer little additional information,  $P=0.4$  in the original 8 class NS-SEC system, or  $P=0.2$  in the recategorised 3 classes. In this case differences in socio-economic status were considered important as they could theoretically obscure a real difference in the outcome, and therefore needed to be accounted for. Thus, inclusion of covariates in adjustment for confounding, as described on p111, were selected on the basis of prognostic strength of the variable not significant tests for baseline differences (de Boer et al. 2015). Thirdly, the balance on reported variables is only assessed by the univariate method. However, pertinent demographic and clinical variables are not completely independent one from another, therefore, presumed balance between groups in individual variables does not necessarily equal the overall balance between intervention groups on all pertinent clinical variables (Festic et al. 2016).

For these reasons the CONSORT guidelines (2010) advise against tests of baseline differences, stating that they are illogical, superfluous and can be misleading. Whilst asking authors to adhere to the CONSORT statement, some reputable journals (New England Journal of Medicine) still instruct their authors to state significant differences

between or among groups (i.e.  $P < 0.05$ ) by identification in a table footnote. Thus, for table 6,  $P > 0.2$  for all variables except disease duration ( $P = 0.03$ ).



Figure 12. Participant country of origin, with frequency illustrated by font size.

Socio-economic class was assessed using an 8-class system (see Appendix ), which was collapsed into a 3-class system, as presented in Table 6. A difference between groups was observed in the pattern of socio-economic status (SEC), with dominance of SEC class 6: semi routine occupations in Diet1 (mainly care workers) and dominance of SEC class 2: lower managerial and professional occupations in Diet2 (primarily health care professionals and teachers). This difference has the potential for bias towards the alternate hypothesis, as higher socio-economic status, as determined by income and education, has previously been associated with greater adherence to Mediterranean dietary patterns in cohort studies (Bonaccio et al. 2012). Therefore, a conservative approach was taken, with adjustment for socio-economic class in the fully adjusted model to prevent possible overestimation of the treatment effect (de Boer et al. 2015).

Table 6. Baseline characteristics of trial participants according to study group (n= 60), expressed as mean (SD) or count (%).

Characteristic	Diet1 Low saturated fat (n = 31)	Diet2 Mediterranean Portfolio (n = 29)	Total (n = 60)
<b>Age, years</b>	42.8 (7.1)	42.0 (6.5)	42.4 (6.8)
<b>Women (%)</b>	17 (55)	14 (48)	31 (52)
<b>Ethnicity (%)</b>			
White European	13 (42)	11 (38)	24 (40)
Black African	14 (45)	16 (55)	30 (50)
Black Caribbean	2 (6.5)	0 (0)	2 (3)
Asian	2 (6.5)	2 (7)	4 (7)
<b>Disease duration, years</b>	6.6 (3.2)	8.9 (4.5)	7.7 (4.0)
<b>Current CD4, cells/mm<sup>3</sup></b>	546 (204)	616 (210)	580 (209)
<b>Nadir CD4, cells/mm<sup>3</sup></b>	195 (122)	230 (196)	213 (163)
<b>Antiretroviral class (%)</b>			
NNRTI	20 (65)	18 (62)	38 (63)
Boosted Protease inhibitor	11 (35)	7 (24)	18 (30)
Integrase inhibitor	0 (0)	4 (14)	4 (7)
<b>Duration of treatment, years</b>	8.2 (3.9)	7.3 (4.2)	7.8 (4.0)
<b>National Statistics Socio-economic class (%)</b>			
I Managerial & professional	9 (29)	15 (52)	24 (40)
II Intermediate	5 (16)	2 (7)	7 (12)
III Working	17 (55)	12 (41)	29 (48)
<b>Ready to change (%)</b>			
Contemplation/preparation	19 (61)	16 (55)	35 (58)
Action/maintenance	12 (39)	13 (45)	25 (42)

Characteristic	Diet1 Low saturated fat (n = 31)	Diet2 Mediterranean Portfolio (n = 29)	Total (n = 60)
<b>Recruitment centre (%)</b>			
Heartlands	20 (65)	20 (69)	40 (66)
Queen Elizabeth	4 (13)	3 (10)	7 (12)
Coventry	7 (22)	6 (21)	13 (22)
<b>QRISK, % 10-year risk of CVD</b>	3.1 (2.9)	2.8 (2.6)	2.9 (2.7)
<b>Smoking status (%)</b>			
Non-smoker	19 (61)	20 (69)	39 (65)
Ex-smoker	8 (26)	6 (21)	14 (23)
Smoker	4 (13)	3 (10)	7 (12)
<b>Systolic blood pressure, mm Hg</b>	123 (15)	125 (14)	124 (14)
<b>Diastolic blood pressure, mm Hg</b>	78 (12)	78 (9)	78 (10)
<b>Lipid profile, mmol/l</b>			
HDL-cholesterol	1.5 (0.5)	1.5 (0.6)	1.5 (0.5)
LDL-cholesterol	3.9 (0.5)	3.9 (0.6)	3.9 (0.6)
Triglycerides	1.2 (0.5)	1.2 (0.5)	1.2 (0.5)
<b>Body mass index, kg/m<sup>2</sup></b>	27.9 (5.9)	28.1 (5.7)	28.0 (5.7)
<b>Obese, BMI &gt;30 kg/m<sup>2</sup> (%)</b>	8 (26)	7 (24)	15 (25)
<b>Waist circumference, cm</b>	93.4 (12.7)	93.9 (11.4)	93.7 (11.9)
<b>Average daily step counts</b>	(n = 25)	(n = 26)	(n = 51)
	5613 (1763)	6029 (2003)	5825 (1882)
<b>Blood HbA1c, %</b>	(n = 25)	(n = 22)	(n = 47)
	5.3 (0.4)	5.4 (0.3)	5.3 (0.4)
<b>Mediterranean Diet Score, 14-item score</b>	6.8 (2.4)	6.0 (2.3)	6.5 (2.3)
<b>Saturated fat intake, g</b>	24.1 (13.2)	24.3 (16.0)	24.2 (14.5)

## Analysis of outcome data

### *Rationale for analysis*

As a pilot/Phase II trial, the effect size produced is likely to be imprecise with a wide 95% confidence interval due to the small participant numbers. However, presentation of the intervention effect estimate is appropriate for the following reasons:

- Funders will consider the existing evidence about an intervention's potential benefit before initiation of a definitive/Phase III trial (Burke et al. 2014);
- The CONSORT extension acknowledges that pilot trials do sometimes assess potential effectiveness using surrogate outcomes (Eldridge, Chan, et al. 2016);
- It would be an oversight to omit reporting a large effect size, should it occur.

With a sample size of 60, this study was calculated a priori to have 80% power to detect 0.5mmol/l (or 10%) difference in LDL-cholesterol between groups at 6 months at 5% significance, assuming a standard deviation of 0.6 (or 10%). This calculation was conservative and did not allow for the adjustment for baseline values in the analysis, which would increase the power. LDL-cholesterol was selected as the proposed primary outcome due to its dose-dependent link to CVD risk suggesting a causal role in the atherogenesis pathway, in addition to being the most commonly used surrogate marker for CVD in trials, allowing this data to be utilized in meta-analyses. Therefore, initial estimates of the treatment effect for the Mediterranean Portfolio diet are presented, whilst acknowledging that these early results may not be replicated in a larger, definitive trial.

### *Strategy to deal with missing data*

The four-point strategy proposed by White et al (2011) was adopted to resolve the issue of how to perform intention-to-treat analysis in the presence of missing data from participants lost to follow-up (White et al. 2011). (See Appendix 11 for full rationale).

1) *Follow-up all randomised individuals*

Attempts were made to follow-up all randomised individuals, even if they withdrew from allocated treatment. This enabled a strict intention to treat analysis to be conducted at month 6 for the proposed primary outcome, for all participants according to their randomisation group, without the need for imputation of missing data.

2) *Perform a main analysis, valid under plausible assumption*

An 'available case' analysis was conducted for outcomes at month 12, acknowledging the limitations of the assumption that participants were missing at random. Data was missing for 4 of the 7 participants who were lost to follow-up at month 12.

3) *Perform sensitivity analyses*

Sensitivity analyses were performed to explore the impact of departures from the assumption that participants were missing at random. Baseline values were imputed where neither month 12 outcomes nor clinical data were available (for LDL-cholesterol  $n = 4$ ).

4) *Account for all randomised individuals in the sensitivity analyses*

All randomised individuals were accounted for.

### *Statistical analysis plan*

The treatment effect estimate for the performance outcomes was explored using multiple linear regression analysis (ANCOVA). Table 7-Table 12 show three analysis models: the raw data in the unadjusted analysis, adjustment for baseline value as pre-specified in the protocol (Stradling et al. 2016), and the fully adjusted model.

Inclusion of multiple variables was avoided to prevent type 1 error, whilst adjustment for variables known to affect the outcome was performed to improve the power of the trial by improving the precision of treatment effect estimation. The rationale for choice of covariates in the fully adjusted multiple regression model was:

- **Baseline value of the outcome** - to account for the severity of the individual's dyslipidaemia and enable examination of the change in LDL-cholesterol rather than absolute values.
- **Gender + smoking** – as it is standard practice to include variables used for allocation stratification. Smoking was categorised as current versus non/ex-smokers.
- **Socio-economic class** – to prevent confounding, as diet quality is known to be higher in people in the upper social classes and thus would impact blood lipid levels. The influence of socio-economic class on the progression of lipid levels could potentially be compounded by the apparent imbalance in socio-economic class between groups following randomisation of small numbers of

participants. Socio-economic class was recoded into three classes (see Appendix ).

- **Baseline Mediterranean Diet Score** – as a measure of pre-existing diet quality, this can be considered a ‘nuisance factor’, the effect of which must be removed by adjustment (Field 2009), or viewed as a prognostic variable that could confound the results of the trial (de Boer et al. 2015). The influence of pre-existing diet quality in our trial cohort is illustrated in Appendix depicting the negative correlation between Mediterranean Diet Score as a marker of diet quality and LDL-cholesterol at baseline.

#### *Deviations from protocol - statistical*

The pre-specified analysis plan stated adjustment for baseline variable only.

Adjustment for randomisation covariates was overlooked, and adjustment for socio-economic status and baseline Mediterranean Diet Score was added after consideration of the clinical importance. Arguably if the adjustment for socio-economic status was solely on grounds of imbalance, it would be conducted in the sensitivity analysis, but due to the clinical importance it was included in the main analysis. It is proposed that for a future trial, socio-economic status will be included as a stratification factor.



Table 7. Adjusted mean differences for blood lipid profile between groups at 6 and 12-month follow-up.

	Diet1 Low saturated fat		Diet2 Mediterranean Portfolio		Diet1 vs Diet2 Unadjusted		Diet1 vs Diet2 Adjusted for baseline outcome		Diet1 vs Diet2 Fully adjusted	
	Mean (SD)	N	Mean (SD)	n	MD (95%CI)	P	MD (95%CI)	P	MD (95%CI)	P
<b>LDL-cholesterol (mmol/l)</b>										
Baseline	3.91 (0.54)	31	3.88 (0.63)	29						
Month 6	3.90 (0.74)	31	3.49 (0.63)	29	-0.40 (-0.75 to -0.05)	0.03	-0.38 (-0.68 to -0.09)	0.01	-0.48 (-0.78 to -0.18)	0.002
Month 12	3.90 (0.72)	28	3.85 (0.70)	28	-0.04 (-0.42 to 0.34)	0.8	-0.05 (-0.33 to 0.23)	0.7	-0.02 (-0.34 to 0.29)	0.9
<b>Total cholesterol (mmol/l)</b>										
Baseline	5.84 (0.69)	31	5.94 (0.73)	29						
Month 6	5.94 (0.92)	31	5.52 (0.88)	29	-0.42 (-0.89 to 0.05)	0.08	-0.51 (-0.85 to -0.17)	0.004	-0.59 (-0.93 to -0.24)	0.001
Month 12	5.99 (0.93)	28	5.93 (0.95)	28	-0.06 (-0.57 to 0.44)	0.8	-0.23 (-0.56 to 0.11)	0.2	-0.26 (-0.64 to 0.11)	0.2
<b>HDL-cholesterol (mmol/l)</b>										
Baseline	1.46 (0.47)	31	1.50 (0.56)	29						
Month 6	1.47 (0.51)	31	1.52 (0.80)	29	0.05 (-0.29 to 0.39)	0.7	0.002 (-0.14 to 0.15)	1.0	-0.01 (-0.16 to 0.14)	0.8
Month 12	1.43 (0.49)	28	1.55 (0.74)	28	0.12 (-0.22 to 0.46)	0.5	0.05 (-0.07 to 0.17)	0.4	0.01 (-0.12 to 0.14)	0.9
<b>Total cholesterol to HDL ratio</b>										
Baseline	4.26 (0.94)	31	4.37 (1.37)	29						
Month 6	4.30 (0.97)	31	4.06 (1.22)	29	-0.23 (-0.80 to 0.33)	0.4	-0.32 (-0.56 to -0.08)	0.01	-0.37 (-0.62 to -0.13)	0.004
Month 12	4.49 (1.15)	28	4.26 (1.31)	28	-0.23 (-0.89 to 0.43)	0.5	-0.31 (-0.62 to 0.002)	0.05	-0.22 (-0.56 to 0.12)	0.2
<b>Triglycerides (mmol/l)</b>										
Baseline	1.21 (0.48)	31	1.23 (0.51)	29						
Month 6	1.27 (0.59)	31	1.11 (0.40)	29	-0.16 (-0.42 to 0.10)	0.2	-0.17 (-0.39 to 0.04)	0.1	-0.18 (-0.41 to 0.05)	0.1
Month 12	1.46 (0.78)	28	1.16 (0.50)	28	-0.31 (-0.66 to 0.05)	0.09	-0.32 (-0.57 to -0.07)	0.01	-0.29 (-0.57 to -0.02)	0.04
<b>LDL-cholesterol (% change)</b>										
Month 6	0.2 (16.5)	31	-9.3 (14.8)	29	-9.5 (-17.6 to -1.4)	0.02	-9.7 (-17.5 to -2.0)	0.02	-11.9 (-19.8 to -4.0)	0.004

Table 8. Adjusted mean differences for key diet variables between groups at 6 and 12-month follow-up.

	Diet1 Low saturated fat		Diet2 Mediterranean Portfolio		Diet1 vs Diet2 Unadjusted		Diet1 vs Diet2 Adjusted for baseline		Diet1 vs Diet2 Fully adjusted	
	Mean (SD)	N	Mean (SD)	n	MD (95%CI)	P	MD (95%CI)	P	MD (95%CI)	P
<b>Mediterranean Diet Score (14-item)</b>										
Baseline	6.84 (2.38)	31	6.03 (2.28)	29						
Month 6	6.57 (2.90)	30	9.52 (2.16)	27	2.95 (1.58 to 4.32)	<0.001	3.34 (2.00 to 4.69)	<0.001	3.08 (1.62 to 4.53)	<0.001
Month 12	5.73 (2.11)	26	7.11 (2.15)	27	1.38 (0.20 to 2.56)	0.02	1.60 (0.38 to 2.82)	0.01	1.08 (-0.13 to 2.29)	0.08
<b>Portfolio score (%) (not measured at baseline)</b>										
Month 6	13.5 (8.8)	28	59.4 (21.3)	27	45.1 (37.1 to 54.7)	<0.001	NA		43.0 (33.4 to 52.5)	<0.001
Month 12	19.1 (12.6)	26	42.5 (19.2)	27	23.2 (14.4 to 32.4)	<0.001	NA		22.3 (12.9 to 31.6)	<0.001
<b>Vitamin A (µmol/l)</b>										
Baseline	2.0 (0.6)	31	1.8 (0.6)	29						
Month 6	2.1 (0.6)	27	2.0 (0.7)	27	-0.12 (-0.47 to 0.23)	0.5	0.04 (-0.25 to 0.33)	0.8	0.05 (-0.24 to 0.34)	0.7
Month 12	2.2 (0.7)	24	2.1 (0.8)	26	-0.16 (-0.57 to 0.26)	0.4	0.03 (-0.35 to 0.40)	0.9	0.18 (-0.14 to 0.49)	0.3
<b>Vitamin E (µmol/l)</b>										
Baseline	30.7 (6.7)	31	31.4 (7.2)	29						
Month 6	32.8 (7.0)	27	28.5 (6.9)	27	-4.3 (-8.0 to -0.5)	0.03	-4.6 (-7.5 to -1.7)	0.002	-4.8 (-7.9 to -1.7)	0.003
Month 12	33.8 (7.4)	24	29.5 (6.9)	26	-4.4 (-8.4 to -0.3)	0.04	-3.7 (-6.8 to -0.6)	0.02	-2.7 (-6.0 to 0.6)	0.1
<b>Vitamin E: Total Cholesterol</b>										
Baseline	5.3 (0.9)	31	5.3 (1.0)	29						
Month 6	5.6 (1.0)	27	5.2 (1.1)	27	-0.4 (-1.0 to 0.2)	0.2	-0.4 (-0.9 to 0.07)	0.1	-0.3 (-0.8 to 0.2)	0.3
Month 12	5.7 (0.9)	24	5.1 (1.2)	26	-0.6 (-1.2 to 0.01)	0.06	-0.4 (-0.9 to 0.08)	0.1	-0.2 (-0.7 to 0.2)	0.3

Table 9. Adjusted mean differences for cardiovascular risk markers between groups.

	Diet1 Low saturated fat		Diet2 Med Portfolio		Diet1 vs Diet2 Unadjusted		Diet1 vs Diet2 Adjusted for baseline		Diet1 vs Diet2 Fully adjusted	
	Mean (SD)	n	Mean (SD)	n	MD (95%CI)	P	MD (95%CI)	P	MD (95%CI)	P
<b>Apolipoprotein A1 (g/L)</b>										
Baseline	1.59 (0.32)	31	1.65 (0.39)	28						
Month 6	1.63 (0.37)	27	1.61 (0.43)	27	-0.01 (-0.23 to 0.21)	1.0	-0.05 (-0.15 to 0.05)	0.3	-0.05 (-0.15 to 0.06)	0.4
Month 12	1.58 (0.34)	24	1.65 (0.48)	25	0.09 (-0.16 to 0.33)	0.5	-0.003 (-0.10 to 0.10)	0.9	0.003 (-0.11 to 0.12)	1.0
<b>Apolipoprotein B (g/L)</b>										
Baseline	1.09 (0.18)	31	1.08 (0.15)	28						
Month 6	1.10 (0.21)	27	0.99 (0.17)	27	-0.11 (-0.21 to -0.001)	0.05	-0.10 (-0.19 to -0.02)	0.02	-0.12 (-0.21 to -0.02)	0.02
Month 12	1.12 (0.22)	24	1.06 (0.17)	25	-0.06 (-0.17 to 0.06)	0.3	-0.07 (-0.14 to -0.002)	0.04	-0.06 (-0.14 to 0.01)	0.1
<b>Systolic Blood Pressure (mm of Hg)</b>										
Baseline	122.8 (14.9)	31	124.9 (13.8)	29						
Month 6	126.6 (16.9)	30	121.2 (9.8)	29	-5.4 (-12.6 to 1.9)	0.1	-6.8 (-1.8 to -11.8)	0.008	-7.6 (-12.9 to -2.4)	0.005
Month 12	129.7 (12.9)	25	126.5 (11.8)	24	-3.2 (-10.3 to 3.9)	0.4	-3.5 (-9.4 to 2.5)	0.2	-2.7 (-9.3 to 3.9)	0.4
<b>Diastolic Blood Pressure (mm of Hg)</b>										
Baseline	77.7 (11.7)	31	77.9 (8.8)	29						
Month 6	80.2 (10.1)	30	78.3 (8.2)	29	-1.9 (-6.7 to 2.9)	0.4	-2.0 (-5.8 to 1.8)	0.3	-2.3 (-6.3 to 1.6)	0.2
Month 12	83.0 (11.4)	25	81.9 (7.3)	24	-1.1 (-6.7 to 4.4)	0.7	-0.5 (-4.7 to 3.7)	0.8	0.4 (-4.2 to 5.0)	0.9
<b>QRISK (10 year % risk)</b>										
Baseline	3.1 (2.9)	31	2.8 (2.6)	29						
Month 6	2.9 (2.4)	31	2.5 (2.3)	29	-0.4 (-1.6 to 0.8)	0.5	-0.2 (-0.5 to 0.16)	0.3	-0.2 (-0.5 to 0.1)	0.2
Month 12	3.3 (2.7)	28	2.8 (2.3)	28	-0.5 (-1.8 to 0.9)	0.5	-0.05 (-0.5 to 0.4)	0.8	0.01 (-0.5 to 0.5)	1.0
<b>Non-HDL cholesterol (mmol/l)</b>										
Baseline	4.39 (0.49)	31	4.45 (0.64)	29						
Month 6	4.47 (0.80)	31	4.0 (0.64)	29	-0.47 (-0.85 to -0.09)	0.02	-0.52 (-0.81 to -0.23)	0.001	-0.58 (-0.88 to -0.28)	<0.001
Month 12	4.56 (0.82)	28	4.38 (0.73)	28	-0.18 (-0.60 to 0.23)	0.4	-0.28 (-0.58 to 0.03)	0.07	-0.24 (-0.57 to 0.10)	0.2

Table 10. Adjusted mean differences for arterial stiffness and inflammatory markers between groups.

	Diet1 Reduced saturated fat		Diet2 Mediterranean Portfolio		Diet1 vs Diet2 Unadjusted		Diet1 vs Diet2 Adjusted for baseline		Diet1 vs Diet2 Fully adjusted	
	Mean (SD)	n	Mean (SD)	n	MD (95%CI)	P	MD (95%CI)	P	MD (95%CI)	P
<b>Carotid pulse wave velocity (m/s)</b>										
Baseline	8.0 (3.0)	31	7.51 (1.65)	29						
Month 6	8.19 (1.91)	29	8.14 (1.95)	27	-0.07 (-1.1 to 1.0)	0.9	-0.001 (-1.0 to 1.0)	1	-1.7 (-1.3 to 0.9)	0.8
Month 12	7.81 (1.66)	26	8.33 (2.52)	27	0.52 (-0.72 to 1.75)	0.4	0.08 (-0.7 to 0.8)	0.8	0.46 (-0.99 to 1.91)	0.5
<b>Augmentation index (%)</b>										
Baseline	22.0 (7.4)	31	24.0 (7.1)	29						
Month 6	23.3 (8.2)	29	24.2 (6.6)	27	0.9 (-3.1 to 4.9)	0.7	-0.5 (-3.5 to 2.6)	0.8	0.3 (-2.9 to 3.6)	0.8
Month 12	22.8 (8.0)	26	23.1 (8.3)	27	0.3 (-4.2 to 4.8)	0.9	-0.9 (-4.6 to 2.8)	0.6	-0.5 (-4.6 to 3.6)	0.8
<b>Highly sensitive C-reactive protein (mg/L)</b>										
Baseline	3.2 (3.4)	30	4.25 (5.20)	28						
Month 6	4.3 (7.1)	28	3.64 (2.88)	26	-0.60 (-3.64 to 2.44)	0.7	-1.30 (-3.82 to 1.23)	0.3	-1.35 (-4.04 to 1.34)	0.3
Month 12	3.1 (4.4)	24	3.65 (2.97)	26	0.64 (-1.51 to 2.79)	0.6	0.11 (-1.88 to 2.09)	0.9	-0.03 (-2.14 to 2.08)	1.0
<b>CD4 (cells/mm3)</b>										
Baseline	546 (205)	31	616 (210)	29						
Month 6	609 (242)	29	609 (226)	28	-0.08 (-125 to 124)	1.0	-44 (-142 to 53)	0.4	-66 (-171 to 39)	0.2
Month 12	594 (265)	22	607 (235)	22	13 (-140 to 165)	0.9	-60 (-143 to 23)	0.2	-79 (-164 to 7)	0.07
<b>Fasting glucose (mmol/l)</b>										
Baseline	4.9 (0.4)	30	5.0 (0.5)	28						
Month 6	4.8 (0.5)	30	5.0 (0.7)	29	0.24 (-0.10 to 0.58)	0.2	0.17 (-0.09 to 0.42)	0.2	0.16 (-0.10 to 0.42)	0.2
Month 12	4.9 (0.6)	26	5.0 (0.6)	27	-0.01 (-0.35 to 0.33)	1.0	-0.07 (-0.34 to 0.2)	0.6	-0.07 (-0.35 to 0.22)	0.6
<b>QDiabetes (% risk)</b>										
Baseline	8.6 (8.7)	31	8.2 (8.7)	29						
Month 6	9.2 (10.3)	31	8.3 (9.0)	29	-0.8 (-5.9 to 4.2)	0.7	-0.3 (-1.2 to 0.5)	0.5	-0.4 (-1.3 to 0.5)	0.4
Month 12	9.8 (10.6)	28	7.8 (8.5)	28	-2.0 (-7.2 to 3.2)	0.4	-0.4 (-1.4 to 0.7)	0.5	-0.3 (-1.4 to 0.9)	0.6

Table 11. Adjusted mean differences for quality of life and gut function between groups.

	Diet1 Reduced saturated fat		Diet2 Med Portfolio		Diet1 vs Diet2 Unadjusted		Diet1 vs Diet2 Adjusted for baseline		Diet1 vs Diet2 Fully adjusted	
	Mean (SD)	n	Mean (SD)	n	MD (95%CI)	P	MD (95%CI)	P	MD (95%CI)	P
<b>Warwick &amp; Edinburgh Mental &amp; Wellbeing score</b>										
Baseline	53.0 (8.2)	31	52.7 (8.1)	28						
Month 6	51.4 (12.8)	27	54.9 (9.3)	26	3.48 (-2.72 to 9.68)	0.3	3.22 (-2.14 to 8.59)	0.2	2.57 (-3.37 to 8.50)	0.4
Month 12	53.7 (11.7)	24	56.0 (8.5)	27	2.30 (-3.39 to 7.99)	0.4	1.76 (-2.93 to 6.45)	0.5	2.87 (-1.82 to 7.56)	0.2
<b>EQ-5D index</b>										
Baseline	0.9 (0.1)	31	0.9 (0.2)	28						
Month 6	0.9 (0.1)	27	0.9 (0.2)	26	-0.02 (-0.10 to 0.07)	0.7	-0.01 (-0.07 to 0.05)	0.7	-0.01 (-0.07 to 0.06)	0.9
Month 12	0.9 (0.2)	24	0.9 (0.2)	27	-0.01 (-0.11 to 0.08)	0.8	-0.01 (-0.09 to 0.06)	0.7	0.004 (-0.08 to 0.09)	0.9
<b>EQ-5D Visual Analogue Scale</b>										
Baseline	77.1 (17.4)	31	79.5 (17.7)	28						
Month 6	79.7 (15.3)	26	86.0 (12.7)	26	6.31 (-1.51 to 14.12)	0.1	4.89 (-1.12 to 10.90)	0.1	4.73 (-2.04 to 11.50)	0.2
Month 12	83.9 (13.9)	24	87.0 (13.2)	27	3.14 (-4.51 to 10.79)	0.4	2.24 (-3.76 to 8.25)	0.5	3.96 (-2.65 to 10.56)	0.2
<b>Gut problems</b>										
Baseline	4.86 (4.42)	28	3.52 (4.57)	29						
Month 6	4.43 (5.09)	28	4.04 (3.81)	27	-0.58 (-3.10 to 1.94)	0.6	0.24 (-1.90 to 2.38)	0.8	-0.63 (-2.93 to 1.66)	0.6
<b>Bristol stool chart score</b>										
Baseline	3.4 (1.6)	27	3.4 (1.2)	29						
Month 6	3.3 (1.2)	28	3.7 (1.4)	26	0.4 (-0.3 to 1.1)	0.3	0.4 (-0.3 to 1.1)	0.2	-0.2 (-0.9 to 0.5)	0.6
<b>Bowel frequency</b>										
Baseline	2.9 (0.9)	28	3 (0.7)	29						
Month 6	2.9 (0.8)	28	3.1 (1.1)	26	0.2 (-0.3 to 0.8)	0.4	0.2 (-0.2 to 0.7)	0.3	0.07 (-0.4 to 0.6)	0.8

Table 12. Adjusted mean differences for anthropometrics between groups at 6 and 12-month follow-up.

	Diet1 Reduced saturated fat		Diet2 Mediterranean Portfolio		Diet1 vs Diet2 Unadjusted		Diet1 vs Diet2 Adjusted for baseline variable		Diet1 vs Diet2 Fully adjusted	
	Mean (SD)	n	Mean (SD)	n	MD (95%CI)	P	MD (95%CI)	P	MD (95%CI)	P
<b>Waist circumference (cm)</b>										
Baseline	93.4 (12.7)	29	93.9 (11.4)	29						
Month 6	92.7 (14.1)	28	92.6 (10.6)	26	-1.31 (-7.95 to 5.33)	0.7	-1.05 (-3.41 to 1.31)	0.4	-1.28 (-3.94 to 1.39)	0.3
Month 12	93.1 (12.7)	23	94.4 (12.0)	23	-0.20 (-7.52 to 7.12)	1.0	-1.66 (-4.37 to 1.06)	0.2	-1.70 (-4.77 to 1.37)	0.2
<b>Weight (kg)</b>										
Baseline	77.9 (15.2)	31	78.9 (16.0)	29						
Month 6	78.1 (16.9)	30	78.9 (16.8)	28	0.7 (-8.1 to 9.6)	0.9	0.18 (-1.45 to 1.82)	0.8	-0.10 (-1.91 to 1.71)	0.9
Month 12	80.4 (18.1)	26	79.6 (17.7)	28	-0.8 (-10.6 to 9.0)	0.9	0.05 (-2.31 to 2.41)	1.0	0.08 (-2.53 to 2.70)	1.0
<b>Body Mass Index (kg/ht2)</b>										
Baseline	27.9 (5.9)	31	28.1 (5.7)	29						
Month 6	27.7 (6.5)	30	27.9 (5.7)	28	0.14 (-3.07 to 3.34)	0.9	0.13 (-0.48 to 0.75)	0.7	0.02 (-0.66 to 0.70)	1.0
Month 12	28.0 (7.2)	25	28.3 (6.0)	27	0.28 (-3.40 to 3.97)	0.9	-0.12 (-1.03 to 0.80)	0.8	-0.14 (-1.17 to 0.88)	0.8
<b>Visceral fat (scale 0 to 60)</b>										
Baseline	8.6 (3.4)	31	8.3 (3.3)	29						
Month 6	8.3 (4.0)	29	8.5 (3.4)	27	0.17 (-1.84 to 2.18)	0.9	0.47 (-0.26 to 1.21)	0.2	0.35 (-0.46 to 1.17)	0.4
Month 12	9.5 (3.9)	25	8.5 (3.0)	27	-1.04 (-2.99 to 0.91)	0.3	-0.46 (-1.44 to 0.51)	0.3	-0.26 (-1.35 to 0.83)	0.6
<b>Subcutaneous fat (%)</b>										
Baseline	30.8 (12.5)	31	30.4 (11.9)	29						
Month 6	28.4 (14.1)	29	30.6 (11.2)	27	2.15 (-4.69 to 8.98)	0.5	1.99 (-0.75 to 4.73)	0.2	1.43 (-1.46 to 4.32)	0.3
Month 12	29.3 (13.8)	25	30.9 (11.8)	27	1.60 (-5.53 to 8.74)	0.7	0.54 (-1.42 to 2.51)	0.6	0.15 (-1.98 to 2.28)	0.9

## Outcome measures

### *Blood lipid profile*

Overall the blood lipid profiles deteriorated in the low saturated fat group (Diet1) during the 1-year follow up with increases in mean total cholesterol, triglycerides, and total to HDL-cholesterol ratio. Conversely there were improvements in lipid levels in the Mediterranean Portfolio group (Diet2) with reductions in mean total cholesterol, LDL-cholesterol, triglycerides, total to HDL-cholesterol ratio, and non-HDL cholesterol (see Figure in Appendix ).

At 6 months, Mediterranean Portfolio Diet2 participants (n = 29) showed a greater reduction in LDL-cholesterol (mean difference adjusted for baseline -0.4mmol/l, 95%CI -0.7 to -0.1, P = 0.01), total cholesterol (-0.5mmol/l, -0.9 to -0.2, P <0.01), total cholesterol to HDL ratio (-0.3, 95%CI -0.6 to -0.1, P = 0.01), non-HDL cholesterol (-0.58mmol/l, 95%CI -0.88 to -0.28, P <0.001), and Apolipoprotein B (-0.1g/L, 95%CI -0.2 to -0.02, P = 0.02) than those in Diet1 low saturated fat group (n = 31). There was no change in HDL-cholesterol, or Apolipoprotein A1, and the -0.1mmol/l reduction observed in triglyceride levels in Diet2 (Mediterranean Portfolio) did not produce a significant difference between groups (see Table 7). In relative terms, the Mediterranean Portfolio diet produced a mean difference between groups at month 6 of 10% reduction in LDL-cholesterol (95%CI -2 to -17, P = 0.02). Figure 13 shows the individual variation in LDL-cholesterol levels. At 12 months, the mean difference between groups was statistically significant for triglycerides (-0.3, 95%CI -

0.6 to -0.1,  $P = 0.01$ ), primarily due to an increase in levels in Diet1 (low saturated fat) participants. All other lipid measures returned towards baseline values, resulting in no significant difference between groups (see Table 7).

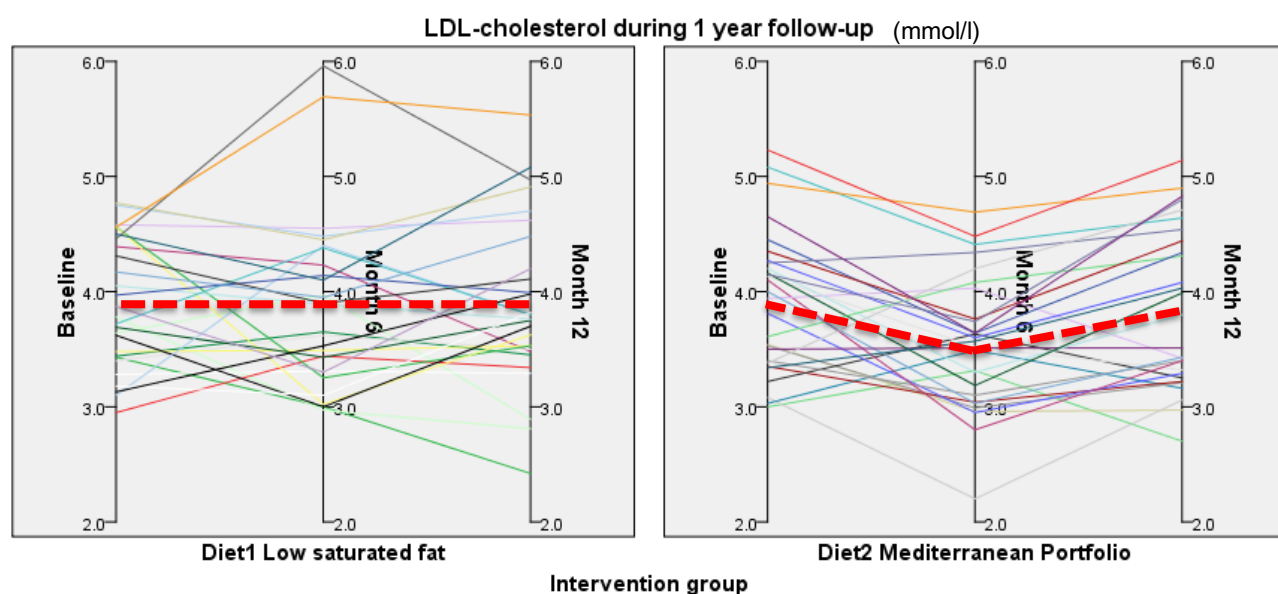


Figure 13. Individual participant levels of LDL-cholesterol (mmol/l) during 1-year follow-up, by intervention group (mean value represented by dotted red line).

### *Cardiovascular Risk Factors*

The mean systolic blood pressure increased in the low saturated fat group (Diet1) and decreased in the Mediterranean Portfolio group (Diet2), producing mean difference between groups -7mmHg (95% CI -2 to -12,  $P < 0.01$ ) at month 6, adjusted



for baseline value. This improvement was attenuated over time, with no significant difference observed at month 12.

The baseline QRISK score was very low, with mean 10-year risk of CVD below 5%, and no individual participant exceeding a risk of 10%. The data did not follow a normal distribution; median QRISK 2.0 (IQR 3.2) in low saturated fat group (Diet1), and 1.8 (IQR 3.2) in Mediterranean Portfolio group (Diet2). QRISK was transformed into natural logarithm to reduce positive skew, and data are reported untransformed in Table 9 for clarity. No difference was observed between groups for QRISK or diastolic blood pressure (see Table 9).

Regarding arterial stiffness, no significant difference was observed between groups in carotid pulse wave velocity or augmentation index (see Table 10).

#### *Mediterranean Diet Score*

Overall diet quality, as assessed by Mediterranean Diet Score (Appendix ), improved by 3.5 points on average. At 6 months, participants in Mediterranean Portfolio (Diet2, n = 29) reported a 3-point greater increase in Mediterranean Diet Score (3.3, 95%CI 2.0 to 4.7, P <0.001) than those in low saturated fat (Diet1, n = 31). At 12 months, eating patterns relapsed such that the difference between groups was halved. Despite this regression, overall improvement in diet quality was maintained by 1.5 Mediterranean Diet Score points at 1 year (1.5, 95%CI 0.3 to 2.7, P <0.02). The individual variation and pattern of specific individuals' improvement in diet quality and

subsequent regression during the trial can be seen clearly in the coloured lines of Figure 14.

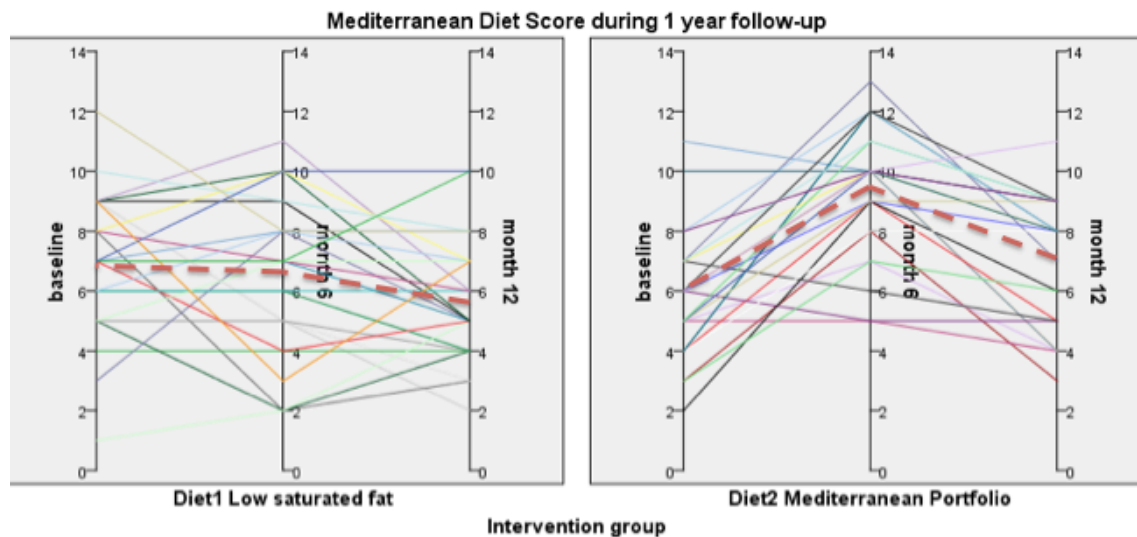


Figure 14. Individual Mediterranean Diet Scores during 1-year follow-up (14-item score, with mean value represented by dotted red line).

#### *Nutrient intake*

Linear regression adjusted for baseline value showed that dietary intakes of energy, protein, carbohydrate, fat, saturated fat, monounsaturated fat, polyunsaturated fat, trans fat, cholesterol, sodium, potassium, retinol and vitamin E were not significantly different between the two groups at month 6 (see Table 13). Trends were observed in the Mediterranean Portfolio group for an increase in proportion of calories from protein and reduction from carbohydrate ( $P = 0.06$ ). A difference was observed between groups for dietary fibre intake ( $P = 0.04$ ) accounted for by the increase of 7g per day in the Mediterranean Portfolio group during the first 6-months ( $P = 0.03$ ).

Dietary fibre was defined as including non-starch polysaccharides, lignin and resistant starch, as the dietary analysis software used quantities that were determined from foods using the American Association of Analytical Chemists (AOAC) method. This AOAC method involves enzymic hydrolysis followed by high performance anion exchange chromatography to identify different fibre fractions.

Looking specifically at mean levels of fat soluble vitamins at month 6 for the Mediterranean Portfolio group, daily intake of vitamin E (7mg) was found to be adequate, exceeding UK requirements of 4mg, but not the Recommended Daily Amount set by the US Food and Nutrition Board of 15mg. For vitamin A, using the currently accepted conversion factor for beta-carotene to retinol of 1:12 (US Food and Nutrition Board), mean intake of 500ug was below the amount recommended by UK Dietary Reference Value of 700ug (Committee on Medical Aspects of Food and Nutrition Policy 1991). As seen in Table 13, the Mediterranean Portfolio group demonstrated a significant reduction in retinol intake ( $P = 0.03$ ), and conversely a trend towards increasing beta-carotene intake ( $P = 0.07$ ) over the 6-month follow-up. There was an opposing trend towards reduction of beta-carotene intake in the low saturated fat group ( $P = 0.07$ ) producing a significant difference between groups at month 6 ( $P = 0.01$ ).

Table 13. Daily nutrient intake of trial participants at baseline and 6 months

After randomisation to one of two diets, expressed as mean (SD) or mean (95%CI).

Nutrient	Low saturated fat group			Mediterranean Portfolio group			Mean difference between groups	P
	Baseline (n = 31)	Month 6 (n = 26)	Within group change*	Baseline (n = 29)	Month 6 (n = 21)	Within group change*		
<b>Energy (kcal)</b>	1905 (887)	1964 (566)	82 (-219 to 383)	1801 (579)	1882 (773)	62 (-200 to 323)	29 (-303 to 360)	0.9
<b>Protein (%)</b>	18.0 (5.1)	18.1 (4.8)	0.3 (-1.9 to 2.6)	17.8 (4.7)	19.8 (4.1)	1.9 (-0.04 to 3.9)	1.9 (-0.5 to 4.3)	0.1
<b>CHO (%)</b>	49.3 (9.1)	48.3 (10.8)	-1.5 (-6.0 to 3.1)	49.1 (6.4)	44.7 (10.9)	-5.4 (-11.0 to 0.2)	-4.3 (-11.0 to 2.4)	0.2
<b>Fat (%)</b>	34.7 (5.6)	33.4 (7.3)	-1.3 (-4.5 to 1.8)	34.7 (14.3)	33.0 (6.9)	-1.7 (-8.3 to 5.0)	-0.3 (-4.5 to 3.8)	0.9
<b>Saturated fat (%)</b>	10.7 (3.4)	10.6 (3.8)	-0.5 (-2.3 to 1.3)	11.4 (4.9)	9.2 (2.7)	-2.2 (-4.7 to 0.4)	-1.5 (-3.5 to 0.5)	0.1
<b>MUFA (%)</b>	10.8 (3.5)	9.9 (3.6)	-1.0 (-2.7 to 0.8)	9.7 (2.3)	10.5 (4.4)	0.8 (-1.5 to 3.1)	0.8 (-1.6 to 3.2)	0.5
<b>PUFA (%)</b>	5.6 (2.4)	5.2 (1.8)	-0.3 (-1.6 to 0.9)	5.3 (1.8)	5.4 (2.4)	0.1 (-1.0 to 1.2)	0.2 (-1.1 to 1.4)	0.8
<b>Trans fats (g)</b>	1.6 (2.3)	1.2 (1.0)	-0.4 (-1.3 to 0.5)	1.0 (0.7)	0.8 (0.7)	-0.3 (-0.8 to 0.2)	-0.4 (-0.9 to 0.2)	0.2
<b>Cholesterol (mg)</b>	267 (267)	231 (136)	-36 (-137 to 63)	253 (138)	203 (110)	-50 (-136 to 35)	-26 (-98 to 46)	0.5
<b>Fibre (g)</b>	18.9 (9.0)	18.0 (7.9)	-0.9 (-4.0 to 2.2)	16.0 (7.6)	22.9 (15.6)	<b>6.9 (0.6 to 13.2)</b>	6.8 (0.5 to 13.2)	<b>0.04</b>
<b>Sodium (mg)</b>	2217 (1781)	2179 (1178)	-180 (-1014 to 653)	1870 (804)	1841 (946)	-171 (-642 to 299)	-322 (-969 to 325)	0.3
<b>Potassium (mg)</b>	2764 (1140)	2557 (684)	-298 (-807 to 211)	2572 (925)	2853 (1921)	215 (-664 to 1094)	414 (-428 to 1256)	0.3
<b>Retinol (ug)</b>	196 (195)	186 (142)	-33 (-105 to 39)	280 (262)	141 (118)	<b>-176 (23 to 329)</b>	-74 (-157 to 8)	0.08
<b>Carotene (ug)</b>	4436 (5961)	2072 (1699)	-2364 (-4912 to 184)	2618 (2260)	4321 (4084)	1859 (-195 to 3913)	2441 (577 to 4305)	<b>0.01</b>
<b>Vitamin E (mg)</b>	6.7 (4.3)	7.3 (3.9)	-0.5 (-2.7 to 1.8)	6.6 (3.4)	7.3 (6.5)	0.2 (-3.0 to 3.4)	0.5 (-2.7 to 3.8)	0.7

Key: CHO carbohydrate, MUFA monounsaturated fats, PUFA polyunsaturated fats, **p<0.05** shown in bold

\* Within group change estimated using paired t tests; Mean difference estimated using linear regression adjusted for baseline value

### *Anthropometric measures*

There was no evidence of difference between groups at month 6 or month 12 in body weight, body mass index, waist circumference, visceral fat, or subcutaneous fat (Table 12).

### *Inflammatory markers*

Levels of the inflammatory marker highly sensitive C-reactive protein were found to be high in both groups ( $>3\text{g/L}$ ). Statistical testing revealed no significant difference between groups before or after the intervention (Table 10).

### *Glucose tolerance*

Diabetes risk, as indicated by QDiabetes score, was low for all participants ( $<10\%$ ). There was no significant difference evident between groups in QDiabetes score or fasting blood glucose levels (Table 10).

### *Immune function*

No evidence of significant difference between groups in CD4 count (Table 10).

### *Plasma vitamins*

Plasma levels of vitamin A rose insignificantly in both groups over the 12 months, with no evidence of a significant difference between groups (Table 8). One participant in low saturated fat (Diet1) group was found to be deficient in vitamin A

(<1µmol/l) at baseline but normalized at month 6. One participant in Mediterranean Portfolio (Diet2), who consumed daily plant stanols, was classified as deficient at month 6 for both vitamin A and vitamin E (<9.5µmol/l) despite high levels of intake of carotene and vitamin E respectively. Conversely another participant, also consuming daily plant stanols, progressed to excess levels of vitamin A (>3.95µmol/l) and vitamin E (>41.5µmol/l) despite a low retinol intake (50µg) and moderate carotene intake (2600µg), although the accuracy of this individual's food diary would be questionable with the low self-reported calorie intake of 1200kcal per day, given maintenance of a stable weight and BMI 25.

Plasma levels of vitamin E rose in low saturated fat group (Diet1), and fell in Mediterranean Portfolio (Diet2) group, such that the mean difference between groups at month 6 was -4.6µmol/l (95% CI -7.5 to -1.7, P <0.01). This difference disappeared after adjustment for total cholesterol, which as a transporter of tocopherols could impact vitamin E levels (Kayden & Traber 1993). Excess levels of vitamin E (>41.5µmol/l) were evident at baseline (n = 4, 2 from each group), month 6 (n = 6, 1 from Diet2 on daily stanols) and month 12 (n = 5, 1 from Diet2 consuming 3 stanols per week).

### *Gut function*

Stool consistency was assessed using the seven categories from the Bristol stool chart score, ranging from 1 for severe constipation to 7 for severe diarrhoea (see Appendix). At baseline both groups erred slightly towards constipation, with average

Bristol stool chart score of 3 and frequency of opening bowels reported as once a day. At month 6, bowel frequency remained unchanged, whilst consistency improved towards normal function with average Bristol stool chart score of 4 in both groups (Table 11). Two participants declined to complete the gut function questionnaire as they felt that describing stools was offensive.

#### *Physical activity*

Physical activity levels were low as no participant achieved the recommended daily step count of 10,000 steps per day. Mean step counts averaged around half of the recommended amount; low saturated fat group 5,613 steps per day (95%CI 4,886 to 6,341, n = 25) and Mediterranean Portfolio group 6,029 steps per day (95%CI 5,221 to 6,838, n = 26). Activity levels did not differ significantly between groups or at different time points during the trial (Table 6).

#### *Quality of life*

No significant difference was observed between groups for health and wellbeing as measured by the Warwick & Edinburgh Mental Wellbeing score (WEMWBS), or quality of life as assessed by EQ5D index and visual analogue scale (see Table 11). From the visual analogue scale, health related quality of life appeared to improve in both groups over the 1-year trial period, with minor non-significant improvements in the region of 7% in Diet1 (paired t test MD 5.5, 95%CI 0.4 to 11.4, P = 0.06) and 8% in Diet2 (paired t test MD 6.8, 95%CI 1.5 to 12.2, P = 0.01). There was a trend

towards a 3-point improvement in WEMWBS at 1-year from baseline in Mediterranean Portfolio Diet2 (paired t test MD 3.00, -0.01 to 6.01, P = 0.05).

Figure 15 shows a visual representation of the frequency of problems reported in the EQ5D-5L questionnaire; numbers are the percentage of participants selecting that response. The most frequently reported problems were anxiety/depression (52%) and pain/discomfort (32%), at levels higher than those reported in the general population (Feng et al. 2015).

#### Month 18 cross-over

At the end of the one-year follow-up, 26 participants from the low saturated fat (Diet1) arm were eligible for the crossover part of the study. Two participants declined the crossover due to either wishing to focus on weight reduction, or enrolling on another clinical trial, and 24 were given the Diet2 information about the Mediterranean Portfolio diet. Of these, only six engaged with the intervention and opted to receive a delivery of cholesterol-lowering foods. Four participants failed to attend the follow-up appointment at month 18; data on lipid profile was collected from clinical records where available. One participant discontinued the intervention due to a hospital admission for psychosis. At month 18, the Mediterranean Diet Score and LDL-cholesterol remained unchanged in participants from the low saturated fat Diet1 group who had received information on Mediterranean Portfolio diet (see Table 14).



EQ-5D Dimension		Diet1 - Low Saturated Fat			Diet2 –Mediterranean Portfolio		
		Baseline	Month6	Month12	Baseline	Month6	Month12
Mobility	No problems	90	86	80	85	85	92
	Slight problems	10	7	16	7	11	4
	Moderate problems	0	7	4	4	0	0
	Severe problems	0	0	0	0	0	0
	Extreme problems	0	0	0	4	4	4
Self-care	No problems	97	100	100	93	96	100
	Slight problems	3	0	0	7	4	0
	Moderate problems	0	0	0	0	0	0
	Severe problems	0	0	0	0	0	0
	Extreme problems	0	0	0	0	0	0
Usually activity	No problems	97	85	92	86	92	85
	Slight problems	3	11	8	7	8	15
	Moderate problems	0	0	0	7	0	0
	Severe problems	0	4	0	0	0	0
	Extreme problems	0	0	0	0	0	0
Pain/ Discomfort	No problems	65	71	64	68	69	52
	Slight problems	26	18	32	18	23	41
	Moderate problems	6	11	4	11	8	7
	Severe problems	3	0	0	3.6	0	0
	Extreme problems	0	0	0	0	0	0
Anxiety/ Depression	No problems	48	64	60	67	69	74
	Slight problems	48	29	36	25	27	22
	Moderate problems	4	7	0	4	4	4
	Severe problems	0	0	4	4	0	0
	Extreme problems	0	0	0	0	0	0

■ <5% reported problems & >95% reported no problems     
■ 10%-25% reported problems & 75%-90% reported no problems  
■ 5%-10% reported problems & 90%-95% reported no problems     
■ ≥25% reported problems & ≤75% reported no problems

Figure 15. Percentage of population sample reporting problems, by dimension and group.

Table 14. Change between month 12 and 18 for low saturated fat group (Diet1) following Mediterranean Portfolio advice, in crossover part of study.

	Diet1 group after Mediterranean Portfolio advice		Change after 6 months on Diet2 (Paired t test)	
	Mean (SD)	n	MD (95% CI)	P
Mediterranean Diet Score (14 item)				
	6.8 (2.1)	18	0.6 (-0.3 to 1.4)	0.2
Portfolio Score (35 item)				
	10.9 (7.9)	20	4.2 (1.2 to 7.2)	0.008
LDL-cholesterol (mmol/l)				
	3.80 (0.78)	23	0.01 (-0.23 to 0.26)	0.9

The mean increase in Portfolio score of 4 points (95%CI 1.2 to 7.2,  $P < 0.01$ ) to a total of 11 indicated 31% adherence to cholesterol-lowering foods by the Diet1 group at month 18 (Table 14). This improvement was not of the same magnitude seen in the Diet2 group, at either month 6 or 12.

### Sensitivity analysis

Sensitivity analyses were conducted for the proposed primary outcome, LDL-cholesterol. When adjusted for socioeconomic status and baseline Mediterranean Diet Score in addition to the previous adjustments of baseline LDL-cholesterol, gender and smoking status, the mean difference at month 6 in LDL-cholesterol between groups was -0.48mmol/l (-0.18 to -0.78,  $P = 0.002$ ) (Table 7). Certain components of the Mediterranean Diet are ubiquitous in normal diets therefore a

degree of contamination was expected. This was evident with some participants in the low saturated fat group (Diet1) having a high Mediterranean Diet Score. Hence adjustment for baseline Mediterranean Diet Score would balance variation in existing dietary patterns between groups to produce the larger effect size observed. In the fully adjusted model, Mediterranean Portfolio (Diet2) participants demonstrated a 12% greater reduction in LDL-cholesterol than those in low saturated fat Diet1 (-11.9%, -19.8 to -4.0,  $P = 0.004$ ). Following imputation for missing data, there was little difference between results from sensitivity analyses for LDL-cholesterol at month 12 (see Appendix), affirming our confidence in the findings.

### **What sample size should be used for the future RCT?**

To determine estimates of the variability of the outcome measures and markers of CV risk, anthropometric measures and quality of life, including LDL-cholesterol, pulse wave velocity and waist circumference.

### **Estimates of variability of future outcome measures**

Estimates of variability of outcome measures will be required for sample size calculation of future trials. The standard deviations of each performance outcome are shown in Tables 4 to 9 and represent the estimate of variability for future outcome measures.

#### *Future outcome measures: LDL-cholesterol*

The standard deviation for LDL-cholesterol ranged between 0.54 and 0.74mmol/l, depending on the group and time point (Table 7). The mean estimate of variability for LDL-cholesterol was 0.58mmol/l at baseline, confirming the original assumption of the variability in LDL-cholesterol as 0.6mmol/l. Therefore assuming 5% threshold probability for rejecting null hypothesis ( $\alpha = 0.05$ ), 80% power ( $\beta = 0.2$ ), 1 to 1 randomisation, and a meaningful effect size of 0.5mmol/l LDL-cholesterol, the sample size required would be  $n = 24$  in each arm (Calculated using the T statistic and non-centrality parameter on [www.sample-size.net](http://www.sample-size.net)) or  $n = 23$  in each arm (Calculated using [www.openepi.com](http://www.openepi.com)). These calculations confirm that the sample of 60 participants in the current study was adequate to use LDL-cholesterol as the primary outcome to determine a clinically relevant difference in LDL-cholesterol between groups.

#### *Future outcome measures: non-HDL cholesterol*

One of the drawbacks to using LDL-cholesterol as an outcome measure was the requirement for participants to fast for 8 hours prior to the blood test. An alternative outcome measure that would not necessitate fasting is non-HDL cholesterol. Recent clinical guidelines have seen a shift towards the use of non-HDL cholesterol as the treatment goal (JBS3 Board 2014). The mean estimate for variability was 0.6mmol/l at baseline (mean 4.4mmol/l). Assuming  $\alpha = 0.05$ ,  $\beta = 0.2$ , two equal groups, and an effect size of 1mmol/l, the sample size required would be 7 in each arm.

*Future outcome measures: markers of cardiovascular risk*

LDL-cholesterol was chosen as the main outcome measure for the current study due to the evidence linking it to CVD risk and myocardial infarction. Measurement of clinical endpoints are not feasible in this relatively young population due to the years of follow up required before cardiovascular events can be observed. Whilst CVD events in people with HIV infection have been reported to occur at higher rates compared with general populations of similar age (Guaraldi et al. 2011; Triant et al. 2007; Currier et al. 2008), the event rate is still relatively low. Rates of MI have previously been reported to range from 1.1 to 3.5 per 1000 person-years (Triant et al. 2007; Data Collection on Adverse Events of Anti-HIV drugs (D:A:D) Study Group et al. 2010). More recently, this has been found to vary with age with a crude MI event rate of 2.3 per 1000 person-years in those aged 40-45 years, and 6.5 in those aged 60-65 years (Petoumenos et al. 2014). In the absence of hard clinical endpoints, a durable surrogate marker is required. Various markers of cardiovascular risk were considered as future outcome measures; therefore, their variability is reported: mean systolic blood pressure (SD 14.3mm of Hg), mean QRISK (SD 2.7%), and mean pulse wave velocity (SD 1.7m/s). Arterial stiffness was chosen as a strong contender for the primary outcome measure in a future trial, however pulse wave velocity measurements displayed wide variance (SD 3 in Diet1 group) and were not different between groups (remaining around 8m/s). The mean pulse wave velocity (8m/s) was higher than European reference values for 40-49 year and 50-59 year age groups (7 and 7.6m/s respectively) (Reference Values for Arterial Stiffness' Collaboration 2010)

yet below the 10m/s standard cut off value in prediction of CV events (Van Bortel et al. 2012).

#### *Future outcome measures: waist circumference*

Waist circumference has potential as a primary outcome, firstly because earlier patient and public involvement work indicated that patients were interested in reducing their abdominal fat or belly size, and secondly due to the point estimate in this pilot study (-1.3cm, 95%CI -4 to 1cm at month 6, and -1.7cm, 95%CI -5 to 1cm at month 12) being of sufficient magnitude to be of clinical importance. The wide confidence intervals suggest that the current pilot study was underpowered to demonstrate a difference between groups. The large variation between individuals, ranging from minimum waist circumference of 65cm and maximum of 136cm, resulted in SD of 12cm. In calculations this forces the size of the sample required up to 1,514 participants, calculated using 90% power at 5% significance to detect a 2cm difference in waist circumference. RCTs in the non-HIV population recruiting >100 participants have similarly also observed large variation with standard deviation of 10cm (Estruch et al. 2018; Greaves et al. 2015). However, other HIV RCTs have reported smaller variation in waist circumference measurements (with standard deviations of 7cm (Almeida et al. 2011), 8cm (Chotivichien, Arab, Prasithsirikul, Manosuthi, Sinawat & Detels 2016b), and 1.1cm (Fitch et al. 2006). However they contain smaller sample sizes than the current study (n=12 in (Fitch et al. 2006)), tend to be of poor methodological quality (Almeida et al. 2011; Chotivichien, Arab,

Prasithsirikul, Manosuthi, Sinawat & Detels 2016b), and are restricted to individuals with a BMI within the normal range (Almeida et al. 2011; Chotivichien, Arab, Prasithsirikul, Manosuthi, Sinawat & Detels 2016b).

Acknowledging that using a larger sample size in a future trial could potentially reduce variation, selection of SD of 10cm for the power calculation would produce a sample size of 1,052. Optimistic prediction of SD of 8cm, by narrowing the eligibility criteria to include a specific BMI range would reduce the sample size to 674.

#### *Future outcome measures: dietary factors*

Intakes of key individual nutrients were not found to be different between groups, except for dietary fibre. This was likely due to the small sample size. Consideration for a future trial would need to be given as to whether secondary outcomes should include a nutrient such as saturated fat intake, or whether the focus remain on dietary patterns.

#### **Sample size calculation for future trial**

Calculation of a sample size for a future trial would need to account for multiple outcome testing if secondary outcome measures are to be added. Advice from an experienced statistician suggested that for a sample size to be considered moderate, it is usually around 500 participants, and that a future RCT should enrol at least 200 participants. This is consistent with estimations based on conducting a trial with one primary outcome, a sample size of 674 participants would provide 90% power at 5%

significance to detect a 2cm difference in waist circumference, assuming a standard deviation of 8cm.

### Potential clinical effectiveness summary

This pilot study has provided the necessary information to enable a decision on whether and how to proceed with a future trial.

Firstly, the findings show that the intervention was successful in generating changes. Eating behaviour migrated towards a healthier dietary pattern, with a 24% improvement in diet quality over the first 6 months, as shown by a 3-point increase in Mediterranean Diet Score. The Mediterranean Portfolio dietary intervention produced a clinically relevant mean reduction of 0.4mmol/l LDL-cholesterol at month 6. The estimated standard deviation used in the power calculation (0.6) was confirmed by the findings of this pilot study, where the estimate of variability for LDL-cholesterol was 0.6mmol/l at baseline. Therefore, the statistically significant between group difference for LDL-cholesterol at month 6 in both the unadjusted (-0.4mmol/l,  $P = 0.03$ ) and adjusted models (-0.5mmol/l,  $P = 0.002$ ) favouring Mediterranean Portfolio diet indicate that the intervention appears to be of benefit. It would therefore be worthwhile to pursue a definitive RCT to test effectiveness.

Secondly, the formal progression criteria for retention and participant compliance with the trial processes were satisfied, therefore the methods were considered viable and the definitive trial can proceed. Attrition rate was significantly less than the 20%



factored into the sample size calculation, and was found to be random, with similar frequency and reasons between groups. However, the target recruitment rate was not achieved; therefore, modifications will be required to the current protocol for a future effectiveness trial. Reducing the time commitment required by participants during the trial in a bid to encourage busy patients to enrol could enhance enrolment. The recruitment target rate needs to be a lower, more realistic figure.

Thirdly, these findings indicate that a future trial is feasible to implement. Feedback from participants indicated that the trial procedures and design were appropriate. Areas for refinement were identified and suggestions were offered that could be incorporated into the design and delivery of a future trial.

## Process evaluation of the intervention

Process evaluations within trials explore the implementation, receipt and setting of an intervention and help in the interpretation of the outcome results (Oakley et al. 2006).

Process evaluations need to be tailored to the trial, the intervention and the outcomes being studied; however, there is an absence of clear guidance in the literature on how this should be reported (Grant et al. 2013). Current work to extend the CONSORT guidelines to social and psychological interventions will address this issue, when published, by emphasising process evaluation. Meanwhile the process evaluation of this study will be presented as the examination of three aspects described in the Medical Research Council guidance (Moore et al. 2015):

- Implementation: the structures, resources and processes through which delivery is achieved, and the quantity and quality of what is delivered;
- Mechanisms of impact: how intervention activities, and participants interactions with them, trigger change;
- Context: how external factors influence the delivery and functioning of interventions.

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### **3. Process evaluation**

#### *Implementation of intervention: fidelity, dose and reach*

To what extent was the intervention delivered as intended?

To conduct process evaluation to assess fidelity and quality of implementation.

Dose & uptake – What was the uptake of the intervention?

To quantify levels of adherence to the dietary intervention.

Reach - Who receives the intervention?  
Are they representative?

To obtain reliable estimates for recruitment, retention and study attendance compliance.

#### *Mechanisms of impact:*

What were the participant responses to the intervention?

To understand how the intervention works in practice.

#### *Context:*

Did external factors influence effectiveness?

To identify contextual factors associated with variation.

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#### *Section summary:*

Process evaluation explored fidelity, dose, uptake and reach, revealing:

- minor adaptations to the intervention to facilitate access to foods and accommodate individual needs,
- highly variable adherence between 11 and 100% (mean 59±21%), influenced by socio-economic status and confidence to make dietary changes rather than contextual factors,
- a group of participants representative of the patient population,
- positive response to the intervention, not intrusive to daily living, with indications of long-term behaviour change.

## Implementation of intervention

An intervention may be falsely deemed ineffective when the structures and resources in place are inadequate to achieve successful implementation. Therefore, it is critical to capture the implementation of the intervention during the trial. Historically assessment of implementation focused on what was delivered, but the more recent implementation frameworks advocate understanding *how* implementation in context is achieved, as well as *what* is delivered, to interpret implementation. This will be presented as fidelity, dose and reach, as recommended by MRC (Linnan & Steckler 2002).

### Fidelity – To what extent was the intervention delivered as intended?

Fidelity refers to the quality of the implementation of the intervention and is of paramount importance in the delivery of dietary interventions. This will be presented in terms of what was delivered (intervention design, technical aspects of delivery, core components), and how implementation was achieved (staff qualifications, quality and use of materials, dosage administered).

### *Intervention design*

The intervention design was described in the methods chapter and remained largely unchanged. Number and frequency of telephone follow-up was diminished due to the difficulty of achieving a connection with the participant rather than their answerphone, and the subsequent time implications for the researcher.

### *Technical aspects of delivery*

The various aspects of the dietary advice were delivered at the separate time points as indicated in the protocol. At the outset of the trial, the intention was for participants to collect supplies of foods at their study visits. It rapidly became apparent that this was impractical for both researcher, maintaining stock levels particularly for refrigerated items, and participant, with issues of transporting multiple heavy bags on public transport. This necessitated an adaptation for the practical implementation of the intervention, by organising supermarket deliveries to the participants' homes. Deliveries direct from the store, at a time convenient to the participant enabled the delivery of a wider range of food items including frozen items such as soya burgers. As the recruitment and follow up of participants covered a period of 2 years, availability of specific foodstuffs also varied. Some foods were discontinued, such as dried soya mince, whilst new foods became available, such as soya desserts.

### *Core components*

Three core components were specified for the dietary advice intervention:

- Mediterranean style Diet: high in vegetables, fruit, olive oil, fish;
- Cholesterol-lowering foods daily: 57g nuts, 2g plant stanols, 15g soy protein, and 15g soluble fibre (oats, pearl barley, lentils, beans, flaxseed);
- Delivered through a Motivational interviewing style consultation using: readiness to change, decisional balance, reflective listening, identifying

motivators and barriers to behaviour change, participant verbalisation of arguments for change, diminish resistance to change, and individual negotiation of goals.

These components were delivered to every participant randomised to the intervention arm by the same researcher, irrespective of recruitment site; therefore, internal validity was maintained.

Motivational interviewing was identified as the delivery style of choice for this dietary intervention due to the evidence base, discussed in the protocol, and ethos.

Motivational interviewing is not a comprehensive treatment but rather a tool for a particular task: to resolve ambivalence in the direction of change. It is relevant for this setting in HIV due to its effectiveness in systems that have previously relied on authoritarian directing (such as dietary advice), and among the most rejected members of society, having been developed for those dependent on alcohol and drugs, and having a greater effect in ethnic minority groups rather than the majority white population (Hettema et al. 2005). During this study, difficulties arose with motivational interviewing in practice, and these observations were recorded in the researcher's reflective diary. Notes indicated a lack of consistence, with not all of the techniques identified in the core components being used with every participant. These initial concerns were allayed by evidence suggesting that the overall aptitude and appreciation for the philosophy of motivational interviewing is more important than competence in or use of particular techniques (Gaume et al. 2009).

Use of the whole motivational interviewing package proved problematic in two types of scenario. Firstly, with some participants of African origin, who were familiar with the authoritarian medical model, found it difficult to engage in patient-led discussions, verbalised by 'you're the doctor, you just tell me what to do, and I'll do it'. Secondly, where participants did not show any ambivalence to making dietary changes and had indicated as such by scoring 10 on the Likert scale of readiness to change on the Baseline Process evaluation questionnaire. In the pursuit of a good quality therapeutic relationship, the researcher majored on the 'focusing' and 'planning' stages of motivational interviewing rather than 'engaging' and 'evoking'. This action was supported by evidence from the literature that clients who were ready to change prior to the intervention demonstrated adverse outcomes to motivational interviewing, reflecting that it may be more appropriate to match clients to the treatment approach (Rohsenow et al. 2004; Miller & Rollnick 2013). This experience highlighted that motivational interviewing is a method ideally suited for clients who are less ready to change, and that alternative methods might be more appropriate for clients at other stages of change.

#### *Staff training*

The researcher was a registered Dietitian with over 20 years experience in the field of HIV. She had attended a 3-day workshop on motivational interviewing, led by the inventors William Miller and Stephen Rollnick. The intention was for the HIV clinical psychologist to review a sample of audio recordings of consultations to assess the quality of motivational interviewing; this was not feasible due to staff changes.

Evaluation of the fidelity of motivational interviewing will require further consideration for future trials, as there will be different therapists at different recruitment sites. The motivational interviewing treatment integrity code examining the proportion of motivational interviewing consistent responses, including the reflection to question ratio and proportion of open questions during the conversation, will need to be explored further.

#### *Quality and use of materials*

The same resources (information sheets, recipes, websites) were used throughout the study period and are detailed in Appendix 9. Participants offered feedback on perceived usefulness; this is presented in Table 19 and Table 20.

#### *Dosage administered*

When an intervention involves advice, it is important to ascertain the consistency of that advice, or the dose delivered, and whether the same level was given to each individual. This is usually standardized to enable replication in other settings, however, in complex interventions this is not always possible or desirable, as one aspect of complexity is the degree of flexibility or tailoring permitted. Flexibility is innate to dietary intervention, as the goal of nutritional therapy is defined as ‘the collaborative development of an individualized eating plan, to address individual nutrition needs based on personal and cultural preferences, health literacy and numeracy, access to healthful food choices, willingness and ability to make



behavioural changes' (Evert et al. 2013). Therefore the aim was to standardize the function and process of the intervention, not the components themselves, to allow for context-level adaptation (Hawe et al. 2004). For this reason, the intervention was not prescriptive, as goals were negotiated individually, taking into consideration socioeconomic factors and family dynamics. Flexibility with dietary requirements also served to prevent attrition by facilitating positive experiences and encouraging commitment to the long term. A large sample size in the future trials will necessitate multiple dietitians providing the dietary intervention across many locations. The aim will be for skilled implementers to deviate from instructions in response to feedback from participants, while remaining consistent with the theoretical basis of the intervention. In preparation for these future challenges, adaptations were monitored and categorized as 'innovation' (skilled implementers actively attempt to make an intervention better fit their population or setting) or 'drift' (unintentional shortcomings, arising from barriers to full implementation) and are presented in Table 15. These classifications will assist in distinguishing between programme tailoring and poor fidelity (Bumbarger & Perkins 2008).

#### Fidelity summary

The intervention was largely delivered as intended. Minor adaptations were made to the intervention that were generally beneficial and improved provision to the population.

Table 15. Deviations from the protocol indicating innovation or drift.

Protocol deviation	Reason	Innovation	Drift
<b>Reduced intake of nuts</b>	BFF35 unable to chew due to dental work	Nut butters were supplied instead of nuts	
	BFF05 reported excema secondary to nuts		Stopped eating nuts but excema did not resolve
	BFF18 concerned about nut allergy		Nil nuts consumed whilst waiting for allergy referral
<b>Reduced intake of plant stanols</b>	BFF11 reported lactose intolerance	Benecol dairy free drinks provided	
	BFF62 disliked taste of stanol drinks	Plant stanol margarine provided	
<b>Variation in soy protein products</b>	Availability of products fluctuated	Dried soya mince was substituted for frozen soya mince	
<b>Increased variety of food products supplied</b>	Initial supplies: oats, barley, lentils, UHT soya milk, desserts, tofu, dried soya mince	Latter stages: oatcakes, Oatibix, canned beans, Frozen soya products – beans, nuggets, burgers	
<b>Collection of foods</b>	Lack of storage facilities with MIDRU; lack of food options within Trust catering procurement systems; participant feedback, “it feels like coming to Foodbank, coming here”	Arranged supermarket delivery of foods to participant’s home address at a time convenient to them	
<b>Review of quality of motivational interviewing</b>	HIV clinical psychologist resigned		Audio recordings of consultations were not reviewed

### **Dose and uptake – What was the uptake of the intervention?**

Dose is defined as the quantity of the intervention that was implemented in practice (Linnan & Steckler 2002). Ascertaining the dose is essential when establishing the extent to which the outcomes evaluation represents a valid test of whether the selected course of action led to the desired change.

In an ideal world the efficacy of the Mediterranean Portfolio dietary intervention would be assessed using an explanatory RCT, under strict study conditions where all food is provided, and participants' eating is observed for the duration of the study, thus ensuring a high level of adherence. This would be extremely costly and invasive; therefore, we chose to undertake a pragmatic trial measuring the effectiveness of the Mediterranean Portfolio dietary intervention in routine clinical practice, under real-world conditions. It is therefore important to quantify participant levels of adherence to the dietary intervention for several reasons. Firstly, to establish whether the intervention as it stands is viable. Very low levels of adherence might suggest that participants did not understand the advice or were unable or unwilling to implement the dietary advice, and the resulting outcomes would be unrepresentative of the true physiological effect of a Mediterranean Portfolio diet. Secondly, to allow patients to rationalize whether the diet is worth doing; knowing that study participants followed half of the diet and achieved a minor effect, future patients could predict that by following all of the diet they might achieve a greater effect. Thirdly, to understand the setting and applicability of the trial results, allowing readers to make meaningful judgments about transferability to their own context (Wells et al. 2012).

This section aims to quantify the levels of adherence to the trial dietary intervention. Participants were given advice and information on how to make changes towards a low saturated fat diet with/without cholesterol-lowering foods of a Mediterranean style. The degree to which these goals were attained differed between participants due to individual variation. The quantity of the intervention that was implemented in practice will be examined both in terms of nutrients and foods. The subsequent implications of this level of adherence on the estimated treatment effect will then be explored.

#### *Levels of adherence – saturated fat*

Both groups were given advice to reduce their daily intake of saturated fat to <10% of energy intake. To quantify this, participants documented their entire food intake over 3 days in food diaries at three different time points: baseline, month 6 and month 12. The food diaries were analysed using Dietplan software to estimate average daily intake of a range of nutrients; these are presented in Table 13. Participants with a reported baseline energy intake outside realistic ranges (<500kcal/d, n = 0 or >3,500kcal/d, n = 1) are usually excluded from the calculations, as these levels suggest under or over reporting (Rhee et al. 2015). However, the one outlier participant who reported 5,044kcal per day was verified at the first follow-up visit when he brought his meal and weighing scales into clinic to demonstrate the type and quantity of food consumed. The food diaries were also re-analysed by an independent dietitian, who produced similar findings.

Intake of saturated fat was 23g/d and 11% of daily energy intake for both groups at baseline. At month 6 this fell slightly in the Mediterranean Portfolio group to 20g/d and 9% ( $P = 0.1$ ), but there was no evidence of a significant difference in daily saturated fat intake between groups, mean difference 4g/d (95%CI -3 to 11). Month 12 data is not shown due to the low return of food diaries ( $n = 36$ ). Only the Mediterranean Portfolio group achieved the goal for percent saturated fat intake of <10% of energy intake.

#### *Levels of adherence – Portfolio components*

The Forest Plot in Figure 16 illustrates that the intake of Portfolio cholesterol-lowering foods at month 6 was significantly different between groups for each item ( $P < 0.01$ ). At month 6 the mean intake of Portfolio foods was low in the low saturated fat group (Diet1), with less than 2 portions per week of each category: stanols, nuts, soya, oats, beans and fish (Figure 16). Within the Mediterranean Portfolio group (Diet2), mean intake varied between 3 and 5 portions per week of each food; the most frequently consumed items being nuts and plant stanols. On average nuts were consumed 5 times a week by participants in Mediterranean Portfolio group (Diet2), and once a week by low saturated fat group (Diet1). Even this low background intake illustrates the problem of contamination in dietary trials where the 'control' group unknowingly consumes 'trial foods' that are naturally occurring in the diet. The difference between groups was least pronounced in intake of beans and oily fish where low saturated fat (Diet1) consumed half that of the Mediterranean Portfolio group (Diet2) (1.5 portions weekly, compared to 2.9 portions).

Within the Mediterranean Portfolio group (Diet2), individual adherence to the weekly target of 35 portions of Portfolio cholesterol-lowering foods varied between 11 and 100%; with mean adherence  $59 \pm 21\%$ . The mean difference between groups in consumption of cholesterol-lowering foods was 46% (95% CI 37 to 55) at month 6, and 23% (95% CI 14 to 32%) at month 12.

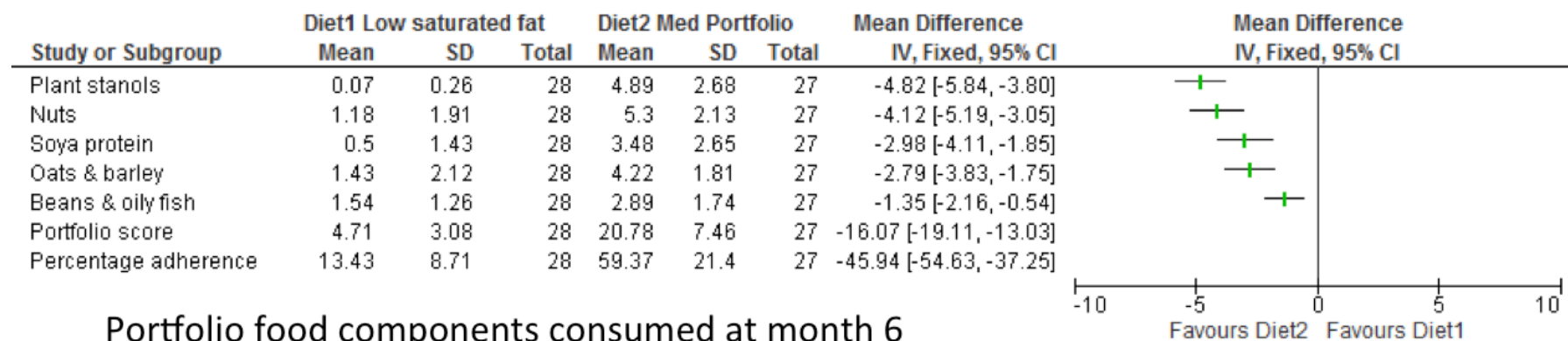
#### *Levels of adherence – Mediterranean Diet*

At baseline, less than a third of study participants exhibited Mediterranean style eating behaviours: only 20% drank more than 2 glasses of wine per week, 23% used more than 4 tablespoons of olive oil per day, 27% ate more than 2 servings of beans or lentils per week, 27% ate more than 2 servings of fish per week, and 28% ate more than 2 servings of nuts per week (Figure 17). The most commonly observed components of the 14-item Mediterranean Diet Score were eating mainly white meat (88% of participants) and using a tomato-based sauce twice a week (73% of participants).

The mean Mediterranean Diet Score rose from 6 at baseline to 10 at month 6 in the Mediterranean Portfolio group (Diet2), whilst remaining stable with a score around 7 in low saturated fat group (Diet1) (see Table 8 on page 119). This change in the Diet2 group towards a Mediterranean style dietary pattern was driven primarily by increased use of olive oil, nuts, and reduced consumption of cake and biscuits, as seen in Figure 17. The proportion of participants achieving dietary goals for intake of cake, nuts, and olive oil as main cooking fat was statistically different between groups

at month 6 (Chi square  $P < 0.01$ ). There was also a statistically significant improvement in the proportion of participants in the Mediterranean Portfolio group (Diet2) who met dietary targets for the consumption of nuts, legumes, fish, and cake at month 6 (Exact McNemar's test,  $P < 0.01$ ). This led to a mean overall adherence to the Mediterranean Diet in the Diet2 group of 68% (95%CI 62 to 74%), as assessed by the 14-item Mediterranean Diet Score.

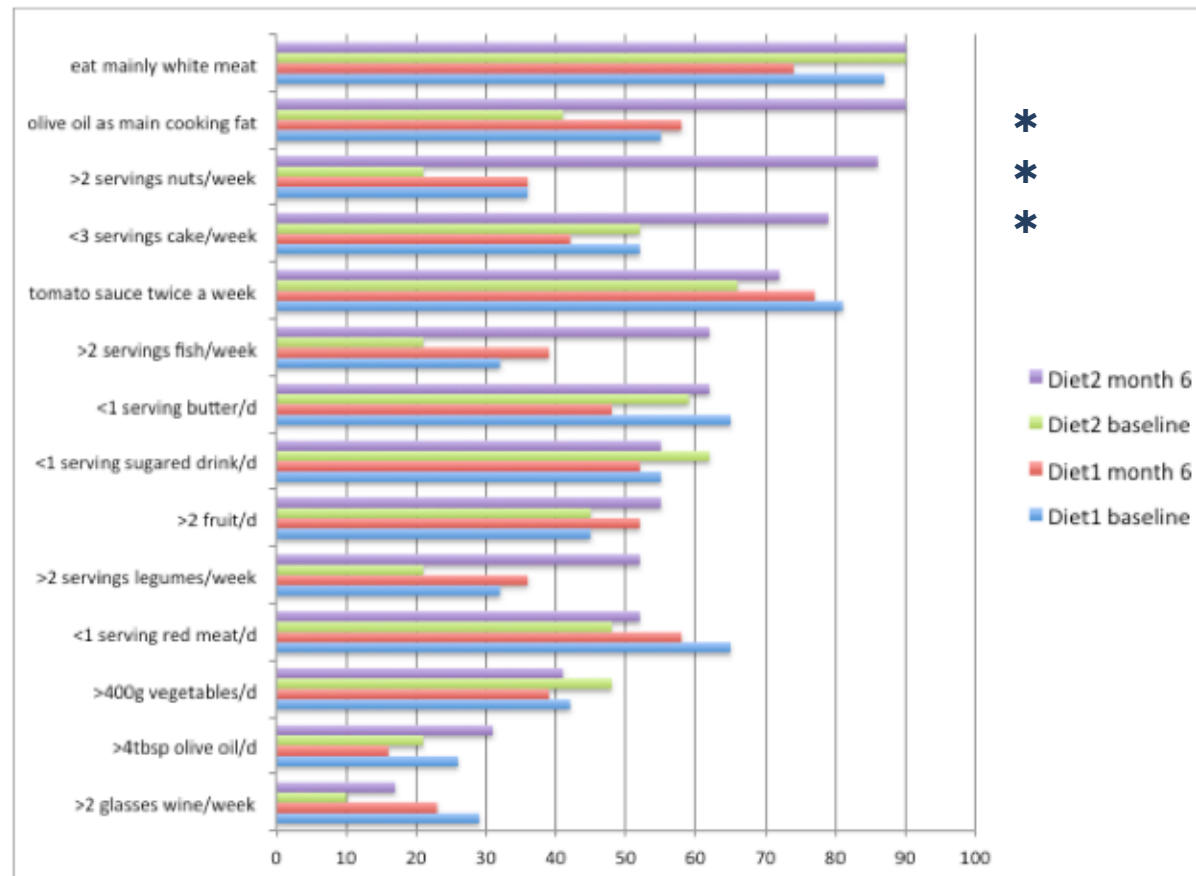
The levels of adherence to the Mediterranean Diet Score that were achieved at month 6 by the Mediterranean Portfolio group (Diet2) were not maintained at month 12. Total Mediterranean Diet Score fell from 10 at month 6 to 6 at month 12, reflecting 43% adherence. Intake of nuts and olive oil were the only components that maintained a statistically significant difference between groups at month 12 (Chi square,  $P < 0.01$ ) (Table 16). Cochran's Q test indicated that there was a difference in the proportion of Mediterranean Portfolio participants achieving targets for legumes, fish, cake, nuts and olive oil as the main cooking fat over the duration of the 1-year study ( $P < 0.001$ ).



Portfolio food components consumed at month 6

Figure 16. Mean intake of Portfolio foods at month 6, by group (35-item score).





Key: \* P<0.05 difference between groups at month 6

Figure 17. Mean percentage adherence to dietary components of the Mediterranean Diet, by group.

Table 16. Number of participants adhering to each of 14 dietary components of Mediterranean Diet Score at baseline, month 6 and month 12, by intervention group, expressed as n (%).

MDS Question	Diet1 – low saturated fat					Diet2 – Mediterranean Portfolio diet					Between groups
Food item	Baseline (n = 31)	Month 6 (n = 29)	Month 12 (n = 26)	§ 6-month change P	¶ Month 12 P	Baseline (n = 29)	Month 6 (n = 27)	Month 12 (n = 26)	§ 6-month change P	¶ Month 12 P	*Month 6 P
Olive oil as main cooking fat	17 (55)	18 (62)	17 (65)	0.4	0.7	12 (41)	26 (96)	23 (89)	<0.001	<0.001	0.002 <sup>^</sup>
>4tbsp olive oil/d	8 (26)	5 (16)	2 (8)	0.5	0.07	6 (21)	9 (31)	6 (23)	0.2	0.2	0.2
>400g vegetables/d	13 (42)	12 (39)	4 (15)	1.0	0.02	13 (48)	12 (41)	6 (24)	1.0	0.07	0.8
>2 fruit/d	12 (45)	16 (55)	7 (27)	0.3	0.08	12 (45)	16 (59)	9 (36)	0.3	0.1	0.7
<1 serving red meat/d	18 (65)	18 (62)	18 (69)	1.0	0.7	13 (48)	15 (56)	12 (46)	0.8	0.7	0.6
<1 serving butter/d	18 (65)	15 (52)	8 (31)	0.6	0.03	16 (59)	18 (67)	10 (40)	0.8	0.09	0.3
<1 serving sugared drink/d	15 (55)	16 (55)	17 (65)	1.0	0.5	16 (62)	16 (59)	15 (58)	1.0	1.0	0.7
>2 glasses wine/week	9 (29)	7 (24)	7 (27)	1.0	1.0	3 (10)	5 (19)	5 (20)	0.6	0.8	0.6
>2 servings legumes/week	8 (32)	11 (38)	6 (23)	0.5	0.3	5 (21)	15 (56)	11 (44)	0.01	0.03	0.2

MDS Question		Diet1 – low saturated fat					Diet2 – Mediterranean Portfolio diet					Between groups
Food item	Baseline	Month 6	Month 12	§ 6-month change P	¶ Month 12 P	Baseline	Month 6	Month 12	§ 6-month change P	¶ Month 12 P	*Month 6 P	
>2 servings fish/week	10 (32)	12 (41)	8 (31)	0.7	0.7	4 (21)	18 (67)	11 (42)	<0.001	0.006	0.058	
<3 servings cake/week	16 (52)	13 (45)	9 (35)	0.6	0.1	15 (52)	23 (85)	15 (58)	0.004	0.2	0.002	
>2 servings nuts/week	11 (36)	11 (38)	6 (23)	1.0	0.2	6 (21)	25 (93)	20 (77)	<0.001	<0.001	<0.001^	
Eat mainly white meat	27 (87)	23 (79)	23 (89)	0.7	0.9	26 (90)	26 (96)	24 (92)	0.6	0.9	0.1	
Tomato sauce twice a week	25 (81)	24 (83)	17 (65)	1.0	0.09	19 (66)	21 (78)	19 (73)	0.3	0.6	0.6	

Key:

d day; tbsp tablespoon; MDS Mediterranean Diet Score

§ McNemar - to test the difference in the proportion of participants achieving the dietary goal after the intervention at month-6

¶ Cochran's Q exact test - to test the difference in the proportion of participants who achieved dietary targets over 1 year

\* Chi-square test - to test the difference between groups at month 6, or 2-sided Fishers Exact test if cells had expected count <5

^ Difference between groups remained p<0.05 at month 12

Despite a stable Mediterranean Diet Score at month 6 for the low saturated fat group (Diet1), the diet quality appeared to deteriorate at month 12 with a reduction in the proportion of participants achieving targets for vegetables and butter (Cochran's Q test,  $P < 0.05$ ). As shown in Table 16, no evidence was found, using Cochran's Q test, for a statistically significant difference in the proportion of participants from either group who met dietary goals for wine, olive oil, red meat, white meat, 'sofrito' (tomato-based sauce), fruit, or sugared drink for over time ( $P > 0.05$ ).

The intake of fruit and vegetables was examined as absolute intake and as categorical intake according to the pre-defined Mediterranean Diet Score questions. After 6 months, in terms of number of servings, 26% (15/57) of participants had increased their daily consumption of fruit (Figure 18), and 23% (15/57) had increased their daily consumption of vegetables (Figure 19). These increases in fruit and vegetables were more evident in the Mediterranean Portfolio group (Diet2). However, when viewed in terms of Mediterranean Diet Score categories there was no difference between the groups, with only 4 participants in each group documenting an increase to  $>400\text{g/d}$  vegetables or  $>2$  servings/d fruit.

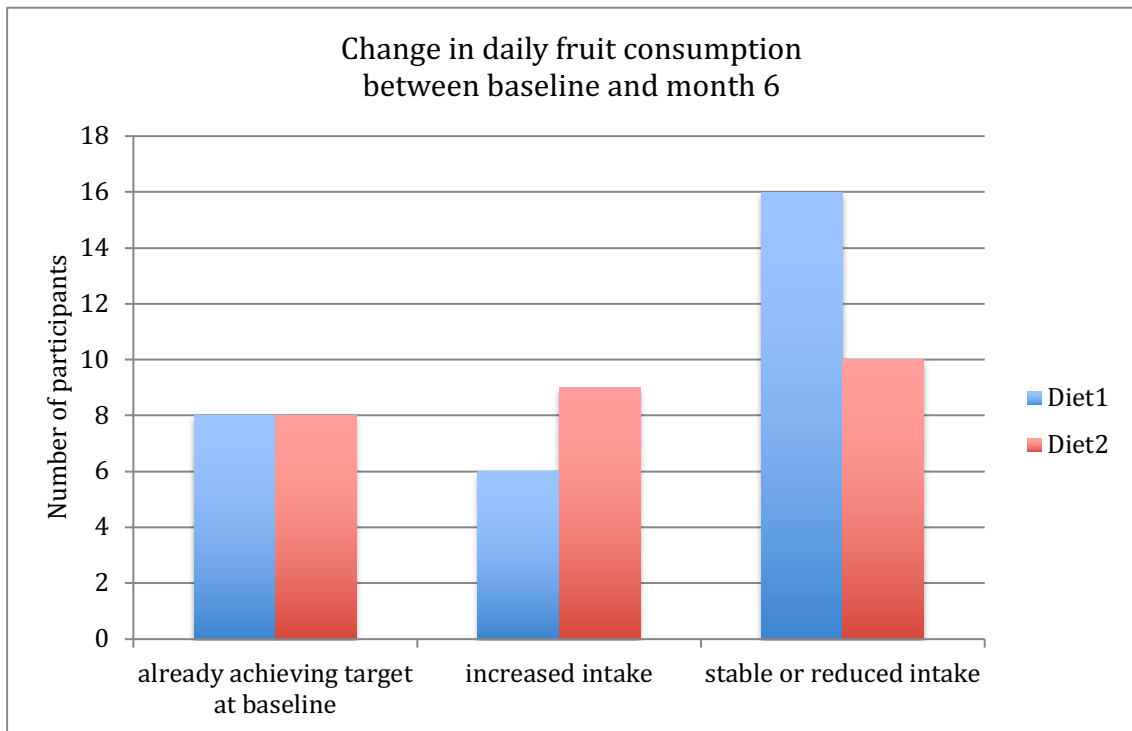


Figure 18. Change in daily fruit consumption between baseline and month 6.

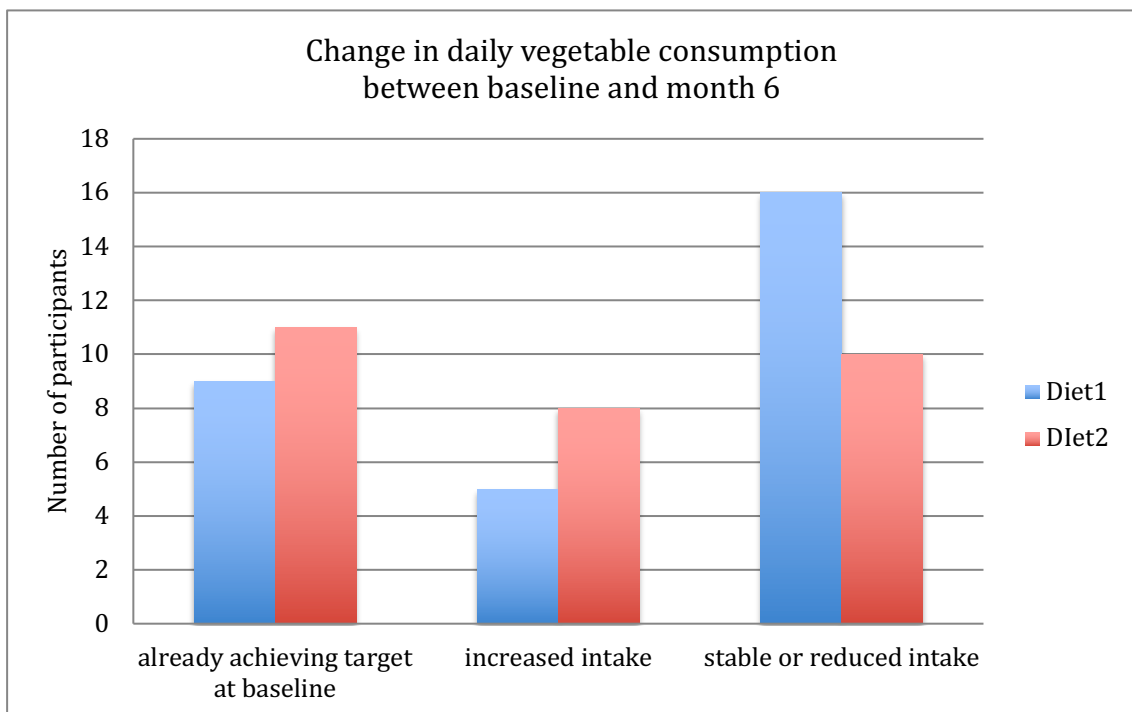


Figure 19. Change in daily vegetable consumption between baseline and month 6.

### Implications of adherence on the estimated treatment effect

The adherence measures reported above indicate that the quantity of the intervention implemented in practice by the Mediterranean Portfolio group (Diet2) was around 60% (mean adherence 59% Portfolio foods, 68% Mediterranean items). To address the issue of non-compliance to the dietary intervention, various alternative statistical analysis methods (Ye et al. 2014) were explored (see Appendix) for detailed explanation). The 'as-treated' approach was performed by regressing the degree of compliance (Mediterranean Diet Score at month 6) on the outcome in a linear regression model; mean difference between groups in LDL-cholesterol -0.43mmol/l (-0.08 to -0.78,  $P = 0.017$ ,  $n = 56$ ). The most commonly used approach is 'per protocol' or 'on-treatment', where participants who did not achieve adequate adherence levels are excluded. Here the regression model was repeated with the 16 participants in Mediterranean Portfolio group who achieved Mediterranean Diet Score 10 and above; mean difference between groups in LDL-cholesterol -0.29 (-0.09 to -0.68,  $P = 0.133$ ,  $n = 45$ ). However, these methods do not account for potential confounding, therefore the Complier average causal effect (CACE) approach is preferred as it estimates the effect of the intervention assuming full compliance whilst preserving the randomisation (Ye et al. 2014). CACE analysis suggests that the reduction in LDL-cholesterol at 6 months might be as great as -0.87mmol/l (95%CI -1.79 to 0.05,  $P = 0.06$ ). This is possibly substantially larger than the fully adjusted ITT effect -0.48mmol/l (-0.78 to -0.18,  $P = 0.002$ ).

## Dose and Uptake Summary

Adherence to the dietary advice was highly variable between individuals. The low saturated fat group failed to achieve the goal for saturated fat intake of <10% of energy from saturated fat. For participants given advice to follow the Mediterranean Portfolio Diet, mean adherence at month 6 was 68% for the Mediterranean Diet, and 59% for the Portfolio cholesterol-lowering foods. Assuming full adherence to the Mediterranean Portfolio diet, Complier average causal effect analysis supports the ITT analysis of a significant treatment effect with a reduction in LDL-cholesterol in the magnitude of 0.4 to 0.9mmol/l after 6 months.

## Reach

As an individual level measure, 'reach' concerns the degree to which (the proportion of) the intended target audience participates in an intervention (Moore et al. 2015). This is usually assessed by attendance, which has been discussed on page 102. For multiple component interventions, the definition has been expanded to include participation in each component (Linnan & Steckler 2002); this has been addressed in page 152 in terms of adherence to the different dietary components. Other exponents of process evaluation see reach as a separate dimension, estimating the extent to which participants are representative of the larger population (Glasgow et al. 1999). This interpretation of reach will be examined in this section, as it can offer key insights into how the intervention might be scaled up after the trial.

### *Who receives intervention?*

The summary characteristics of the 60 patients recruited into the trial are presented in Table 6. Overall, half of the participants were of African ethnicity, and there were nearly equal numbers of men and women. These proportions were mirrored in the intervention group.

### *Do the eligibility criteria select a representative sample of the population?*

During the screening process at Heartlands Hospital, data were collected on subject gender, ethnicity, LDL-cholesterol levels, viral load, current medication and past medical history to ascertain reasons for ineligibility within the cohort. Historically, HIV clinical trials have recruited predominantly white gay men; therefore, participant characteristics were examined during the stages of enrolment to establish whether the trial participants were representative of the patient population. Early conversations during recruitment also revealed a lack of understanding of the Patient Information Sheet with those of African origin, with an expectation that they would be asked to take drugs, in addition to a general distrust in research and the medical system. Half of the patients screened were white European, and the other half predominantly Black African, with a few Black Caribbean (6%) and Asian (3%). Using Chi square test there was no evidence of an association between ethnicity and eligibility ( $P=0.5$ ), or enrolment ( $P=0.4$ ). Post hoc power calculation suggested that there was 90% power to detect a medium to large (effect size, 0.3 to 0.5 Cohen) difference at 5% significance with this sample size. One third of the cohort were



female (292/882, 33%), and the same proportion of women was found to be eligible for the study (48/145, 33%). Using chi square test there was no evidence of an association between gender and eligibility ( $P=1.0$ ). However, significantly more women were enrolled (22/50, 44%,  $P=0.04$ ) than men, because fewer declined (18/68, 26%,  $P=0.1$ ).

In summary, the trial participants were representative of the patient population with respect to gender and ethnicity.

### **Mechanisms of impact: understand how the intervention works in practice**

Participant responses to an intervention begin with the 'dose received', as discussed in section 3.1.2 but how adherence is achieved is more complex, as whether an intervention works or not depends on how participants respond to it. This section explores participants' interaction with the intervention to understand how change is produced in the short and long term.

### **How change is happening**

The Process Evaluation questionnaire at month 6 included a multiple-choice question about general changes that participants had made since being involved in the study. Purchasing more healthy foods was the most frequently reported behaviour change following participation in the trial. Alteration in cooking methods was reported by fewer people. Overall more participants in the Mediterranean Portfolio group (Diet2)

reported making positive dietary changes than those in low saturated fat group (Diet1), as shown in Figure 20.

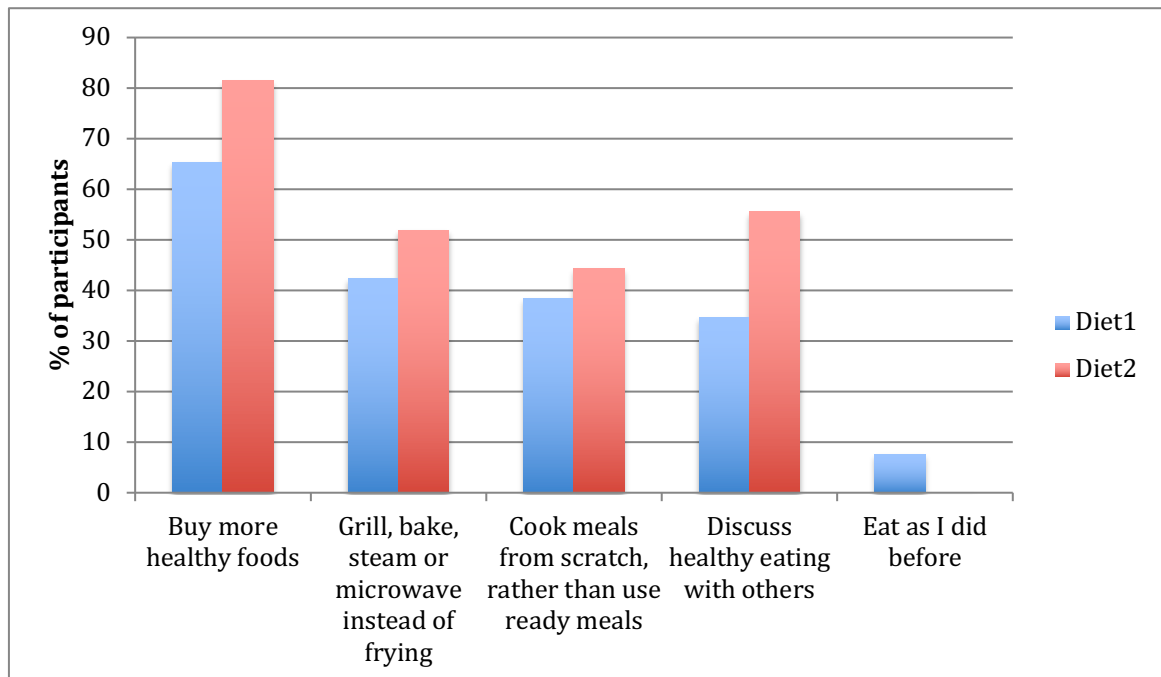


Figure 20. Participant responses to, 'My involvement in the trial has encouraged me to:'

#### Participant responses to intervention – interactions and change

During the interviews with the Mediterranean Portfolio group (n=16), questions were asked about what dietary changes had been made or not.

Participants were keen to talk about the changes they had made to lower their saturated fat intake by reducing quantities of crisps, chocolate, take-aways, chips, pastry, pizza, butter, cake, and mayonnaise. They spoke about how they had incorporated Portfolio foods such as nuts as a snack, sardines on toast, porridge with

linseed for breakfast; also, about wider changes towards a Mediterranean style diet such as stopping carbonated drinks and high sugar breakfast cereals, and changing to brown rice, seeded bread, and olive oil. The transcripts were infused with descriptions of how they had adapted behaviours, choosing salad when eating out, fish instead of steak, frozen yogurt instead of ice cream, checking food labels, removing skin from chicken, cutting fat off meat, and eating a wider variety of vegetables. There was evidence of changes in cooking practice with accounts of making own tomato sauce, adding grated vegetables, linseeds in flapjacks, lentils in casserole, oats in cookies, using olive oil dressings on salads, throw everything into the slow cooker, and tofu in curries. One individual acknowledged that the changes were partial, 'I was still kind of eating crap' (white, male) but sought out snacks containing seeds, lentils, and soya when buying lunch at work.

Foods that were not incorporated into the diet included stanols (3), oats (2), and beans. Soya was excluded in its entirety for 6 participants, but the majority talked about a 'disaster' with one soya food, such as edamame beans (3), soya milk (3), soya mince (2), tofu (2), or meatballs (2), whilst incorporating other types, such as soya mince (5), soya desserts (5), soya milk (3), edamame beans (2), and soya burgers. This highlights the individual variation and importance of offering a selection of foods. Interviews were conducted after trial completion, yet all the foods were mentioned without prompting, demonstrating retained knowledge and understanding of the diet. The exception to this increased knowledge was one participant who described using beans to '*soak up fat from the meat*' and justified the use of palm oil

instead of olive oil. Participants reported that they were previously eating beans/lentils (7), nuts (5), oats/barley (3), fish (3), stanols (2), tomato-based sauce, and olive oil highlighting the baseline intake of 'intervention' foods that could potentially limit the effect size / degree of underlying contamination.

#### **Participant responses to intervention - maintenance**

Two of the Likert style questions in the Process Evaluation Questionnaire at month 6 examined traits suggestive of maintenance of behaviour change. The most common response indicated that participants now kept a record of what they eat and set themselves nutrition goals (mean/median/mode 3, 'agree' with statement). The median score 3 indicated that participants agreed with the statements 'I now keep a record of what I eat', and 'I now set myself nutrition goals'.

#### **Participant responses to intervention – Diet intrusiveness**

Distribution of data from the diet intrusiveness score was positively skewed, therefore median values are presented and differences were estimated using independent samples Mann-Whitney U test. There was no evidence of a difference in distribution of total diet intrusiveness between groups (Diet1 26 IQR 10, Diet2 27, IQR 10,  $P = 0.8$ ). Scores were low indicating that the new way of eating did not interfere with participants' lives (minimum score 20, maximum score 100).

The frequency of intrusiveness scored 13 in each group (minimum score 10, maximum 50), indicating that the new diet was 'never/rarely' a problem for individuals

(Diet1 13, IQR 6, Diet2 13.5, IQR 5,  $P = 0.9$ ). Similarly, scores for impact indicated 'low/moderate' interference with participant's life (Diet1 13, IQR 5, Diet2, 13, IQR 6,  $P=0.8$ ). Of the individual components, 'makes it difficult to be spontaneous' scored highest for both frequency and impact of interference of the new diet with daily life. All other components scored a median of 1, which equated to 'never' and 'low impact'. Outliers with higher scores were identified as individuals who travelled with work (2 African females, 1 African male).

### Unintended consequences

Certain unintended consequences were anticipated, and so measures were put in place to capture potential changes. Quality of life was assessed during the trial to monitor any potential deterioration due to the difficulty of implementing the dietary intervention. No adverse change in outcomes were observed, as described in Figure 15 and Table 11.

Fat-soluble vitamin levels were monitored due to previously documented concerns regarding malabsorption with use of plant stanols. Overall, the Mediterranean Portfolio diet had a beneficial effect on mean plasma levels of vitamin A. However, there was one case of deficiency and one case of excess levels of vitamin A at month 6. Conversely, harmful consequences were observed with a fall in plasma vitamin E levels in the Mediterranean Portfolio diet group. In contrast, there was only one case of deficiency at month 6, but 5 cases of excess levels of vitamin E occurring at baseline (2), month 6 (2) and month 12 (1).

### **Mechanisms of impact summary**

Participants interacted with the intervention by instigating multiple practical dietary changes to both types and quantities of foods. Where one food was disliked, another was substituted, and the intervention was not found to be intrusive to daily living. Overall response to the intervention was positive with indications of long-term behaviour change. The unintended consequences of the Mediterranean Portfolio diet were unclear, with cases of deficiency and excess in levels of fat-soluble vitamins; all of who were consuming plant stanols.

### **To identify contextual factors associated with variation.**

The pre-existing circumstances, attitudes and beliefs of participants will shape how they interact with the intervention, possibly producing variation in outcomes. This section will examine external factors and predictors of adherence to see who benefits from the dietary intervention.

### **Contextual factors - cooking skills**

Regarding cooking skills, most participants described themselves as being comfortable following recipes and creating meals. Only a handful reported having little cooking experience or basic cooking skills, (4 in Diet1, 8 in Diet2). Using Fisher's exact test, no association was found between reported cooking skills and existing diet quality (represented by baseline MDS,  $P = 0.6$ ), or dietary adherence at month 6 (as measured by MDS at month 6,  $P = 1.0$ ).

### Contextual factors – environmental factors

The atmosphere of social support surrounding participants was assessed on the PE1 questionnaire using five questions with a 5-point Likert scale. The first two questions reflected positive social support and the last 3 questions reflected negative social support (minimum score 5, maximum score 25). Mean overall scores were similar in both groups (see Table 17), suggesting that participants received neutral support from family, friends and co-workers.

Table 17. Social support reported by participants, by group.

People who are close to me.....	mean Likert score $\pm$ SD	
	Diet1	Diet2
<b>Encourage me to eat healthy foods</b>	3.5 $\pm$ 1.1	3.3 $\pm$ 1.1
<b>Remind me to eat healthy foods</b>	3.4 $\pm$ 1.2	3.5 $\pm$ 1.1
<b>Criticise me for eating healthy foods</b>	3.9 $\pm$ 1.0	3.5 $\pm$ 1.1
<b>Offer me unhealthy foods</b>	2.8 $\pm$ 1.3	2.8 $\pm$ 1.1
<b>Eat unhealthy foods in front of me</b>	3.0 $\pm$ 1.3	3.1 $\pm$ 1.1
<b>Total score</b>	16.5 $\pm$ 3.2	16.3 $\pm$ 2.2

### Contextual factors – intention to change

Using Rollnick's readiness ruler (Miller & Rollnick 2013), participants scored their intention to make dietary changes on a scale of 1 to 10 as part of the Process

Evaluation questionnaire (PE1) at baseline. The mean scores were high, implying that participants were confident on the why, how, and when of making dietary changes. Using independent t test there was no evidence of a difference between groups in readiness to change (the 'when') (Diet1  $8.1 \pm 2.2$ , Diet2  $8.6 \pm 1.6$ , MD -0.4, 95%CI -1.4 to 0.6,  $P = 0.4$ ), confidence to change (the 'how') (Diet1  $8.2 \pm 2.2$ , Diet2  $8.4 \pm 1.7$ , MD -0.2, 95%CI -1.3 to 0.8,  $P = 0.6$ ), or perceived importance of change (the 'why') (Diet1  $8.3 \pm 2.3$ , Diet2  $8.8 \pm 1.5$ , MD -0.6 95%CI -1.6 to 0.5,  $P = 0.3$ , all  $n = 29$  in both groups. A potential trend was observed towards increased confidence to make dietary changes in those with high adherence ( $P=0.02$ ) see Table 18.

Confidence to change was also assessed by asking participants whether they agreed with several self-efficacy statements previously used in health psychology (M.-W. Chang et al. 2008). Most participants agreed with the statement 'I am certain that I can eat a healthy diet, even if things get difficult' (all participants rated the statement 'agree' or 'strongly agree' on the Likert scale). These findings were similar to the positive agreement given to statements that reflected motivation and outcome expectation such as 'eating low fat foods will...make me feel good'.

### Contextual factors – personal circumstances

Most pre-existing circumstances were captured in one of the assessments, for example one participant's disabilities and wheelchair use was reflected in the EQ5D scores. However, there were instances where circumstances changed during the study that would be likely to affect the outcomes, such as extended travel abroad,



partner and food provider starting Weight Watchers diet, use of 'party' drugs that cause raised heart rate and blood pressure (Mephedrone).

Some participants experienced life events, such as bereavement, divorce, or loss of employment that impacted their ability to implement the diet. Two individuals in the Mediterranean Portfolio group became homeless during the trial, one due to asylum issues, and one due to drug misuse.

### Predictors of adherence

Sub group analysis was used to examine which pre-existing characteristics predict who benefits from the intervention. For example, it was hypothesized that socioeconomic conditions moderate how participants interact with the intervention, and hence its effectiveness. Whilst sub group analyses can be criticized as statistically unsound (Petticrew et al. 2012), they can identify trends, which, while not statistically significant in individual studies, point to meaningful differences if replicated across studies.

The PREDIMED study defined high adherence as MDS>10 because half of participants complied with 11 or more items at each follow-up visit (Downer et al. 2016). However, no evidence was found in the literature for what constituted a 'good' Mediterranean Diet Score, therefore a more conservative threshold for 'high adherence' was determined by using the top tertile of the Mediterranean Portfolio group (Mediterranean Diet Score 10). This top tertile definition has been used in

similar studies (Kyriacou et al. 2015). Chi square tests were used to assess differences in distributions of baseline characteristics between those with low adherence and high adherence. No evidence was found of an association between age, gender, ethnicity, readiness to change, obesity or baseline LDL-cholesterol and high or low adherence to Mediterranean Diet Score at month 6.

To address the question regarding differences in responses to the intervention between subgroups of participants, logistic regression analysis was used to calculate odds ratios of adherence to the Mediterranean Diet at month 6 to identify participants most and least likely to benefit from the intervention (See Table 18). However, these approaches are equivalent to sub set analyses as they ignore the original random assignment to treatment and control groups and disrupt the group equivalence produced by random assignment (Sagarin et al. 2014). Reporting high levels of confidence to make dietary changes was found to be a predictor of adherence to the Mediterranean Portfolio intervention (OR 1.9, 95%CI 1.1 to 3.5,  $P = 0.03$ ). The possibility of an association between socio-economic status and adherence levels requires consideration in a future trial, as a trend was observed towards high adherence in those of higher socio-economic status, classified as managerial, professional and self-employed workers ( $P = 0.05$ ).

Table 18. Predictors of dietary adherence.

Presented as mean  $\pm$ SD, n (% of those with high/low adherence), and odds of high adherence with the Mediterranean diet at month 6 according to specific baseline characteristic (OR <1 imply poorer adherence, OR>1 imply better adherence).

	High adherence (MDS>9)	Low adherence (MDS<10)	P for Chi	Odds Ratio (95% CI) for dietary adherence	P
Number of participants	16 (73%)	13 (37%)		Diet2 participants only	
Age, years	43.3 $\pm$ 7.0	40.4 $\pm$ 5.7	0.2*	1.1 (1.0 to 1.2)	0.2
Male	9 (56%)	6 (46%)	0.6	0.7 (0.2 to 2.9)	0.6
White European	5 (31%)	6 (46%)	0.5	1.9 (0.4 to 8.6)	0.4
Obese BMI>30	3 (23%)	4 (25%)	1.0	0.9 (0.2 to 5.0)	0.9
Current smoker	2 (13%)	1 (8%)	1.0	0.6 (0.05 to 7.3)	0.7
High SEC	12 (75%)	5 (39%)	<b>0.05</b>	0.2 (0.04 to 1.0)	<b>0.05</b>
Duration HIV diagnosis, years	10.0 $\pm$ 4.6	7.7 $\pm$ 4.3	0.2*	1.1 (0.9 to 1.3)	0.2
Years on ART	7.6 $\pm$ 3.8	7.0 $\pm$ 4.7	0.7*	1.0 (0.9 to 1.2)	0.7
Taking Protease inhibitor	4 (31%)	3 (25%)	1.0	0.8 (0.1 to 4.4)	0.7
Baseline Med. Diet Score	6.6 $\pm$ 1.9	5.3 $\pm$ 2.5	0.1*	1.4 (0.9 to 2.0)	0.1
Baseline LDL-cholesterol	3.8 $\pm$ 0.5	4.0 $\pm$ 0.7	0.4*	0.6 (0.2 to 2.0)	0.4
Baseline QRISK	3.0 $\pm$ 2.3	2.6 $\pm$ 3.1	0.7*	1.1 (0.8 to 1.4)	0.7
Recruited at Heartlands	12 (75%)	9 (69%)	1.0	0.8 (0.1 to 3.8)	0.7
Attended all appointments	15 (94)	11 (85)	0.6	0.4 (0.03 to 4.6)	0.4
Importance to change	8.9 $\pm$ 1.5	8.8 $\pm$ 1.5	0.8*	1.1 (0.6 to 1.7)	0.8
Confidence to change	9.1 $\pm$ 1.3	7.6 $\pm$ 1.8	<b>0.02*</b>	1.9 (1.1 to 3.5)	<b>0.03</b>
Readiness to change	8.8 $\pm$ 1.5	8.3 $\pm$ 1.7	0.4*	1.2 (0.7 to 1.9)	0.4
Minimal cooking skills	4 (27%)	4 (31%)	1.0	1.2 (0.2 to 6.3)	0.8

Key:

\* Independent t test for parametric data; Mann Whitney test for non-parametric data  
Fishers Exact test used instead of chi square where cells have expected count <5

### Contextual factors summary

Contextual factors such as cooking skills, readiness to make dietary changes, or belief about importance of the diet, did not appear to influence adherence to the Mediterranean Portfolio dietary intervention. Examination of predictors of adherence highlighted a potential for the influence of socio-economic status and level of confidence to make dietary changes. These pre-existing characteristics require consideration in a future trial and could be used to predict who would benefit from the intervention. Within the wider context, this study was fully funded and conducted within specialised clinics in the NHS setting; this is likely to influence the delivery of the intervention.

## Feasibility of a future trial

### How should a future RCT be performed?

Having ascertained from section 2 that it is worthwhile to undertake a future RCT, and from section 1 that a trial is viable with regards to recruitment and retention, this section examines how a future RCT should be performed. The role of RCTs in establishing causality is dependent on rigorous methodology being employed. Critical features of an RCT include use of a control group to avoid bias due to confounding factors, randomisation to prevent selection bias and minimize confounding due to unequal distribution in the population, and double blinding to prevent interpretation bias. The pilot study was used to test uncertainties in the trial design and this section discusses particular aspects of concern.

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#### ***Section 4: Feasibility of future trial***

Research question	Objective from protocol	Tools used
How should a future RCT be performed?  Is it feasible?	To explore the appropriateness of the trial procedures, design and duration.  To examine acceptability from the participants' perspective.	PE1 allocation preference PE2:1 allocation satisfaction PE2:1 types of support - acceptability PE2:3 strengths/weakness, improvement suggestions Interviews Q6: changes to trial Food diary survey

*Section summary:*

Trial procedures were found to be acceptable from the participants' perspective and appropriate with regards to systematic screening, randomisation, allocation concealment and stratification, resources used to support dietary intervention, outcome measurements, and overall organisation. Paper food diaries were selected in preference to the electronic app MyNetDiary. Suggestions for future trial design and process are proposed.

**Appropriateness of trial design: Screening process**

Screening methods were systematic but varied slightly for each site, to accommodate local practices, capacity of research nurses, and lead consultants' preferences. At Heartlands the researcher screened HIV clinic lists weekly to identify potentially eligible patients, who were given the Patient Information Sheet and opportunity to ask questions about the study. The enrolment appointment was booked to coincide with their next attendance for clinical care. At Coventry the physician gave the Participant Information Sheet to patients during his HIV clinic, and the Comprehensive Local Research Network (CLRN) recruitment facilitator posted food diaries and contacted them to confirm interest and arrange an enrolment appointment with the researcher. At the Queen Elizabeth Hospital HIV clinic, the CLRN recruitment facilitator screened the electronic records for key blood results (LDL-cholesterol, HIV viral load) and contacted patients who were potentially eligible to provide the patient information sheet and ascertain interest before booking an enrolment appointment with the researcher.

Additional ad hoc recruitment methods were also employed via posters in the waiting room, referral from the multidisciplinary team, and enquiries from the trials registry; these methods yielded less than ten subjects, who were all ineligible. It was the systematic recruitment method of screening blood results of patients attending HIV clinics that consistently identified eligible participants.

#### Appropriateness of trial design: randomisation

Ideally in an RCT design, both the participant and researcher should be unaware of which intervention they are receiving in order to reduce the chance of a biased result. This blinding is difficult to achieve in dietary intervention RCTs. To ascertain how participants felt about the random allocation to an intervention group, they were asked prior to randomisation, which diet they preferred to be allocated to, the 'easy old diet' (Diet1 arm) or the 'new complex diet' (Diet2 arm). The actual descriptions of 'low saturated fat' and 'Mediterranean Portfolio' could not be used in order to protect the identity of the intervention and prevent contamination between groups. When Chi Square was tested, no evidence was seen of an association between allocation preference and the actual group individuals were assigned to (Diet1  $P = 0.3$ , Diet2  $P = 0.6$ ). At month 6 the subject was broached again in the process evaluation where 80% of Diet1 participants reported satisfaction with group allocation, and 17% selected the 'don't know' response (see Table 19). In addition, comments from the qualitative interviews also suggested that blinding, although not planned, was achieved,

*'I went into it with absolutely no real expectations. I knew there were two different diets, I had no idea what they were; what changes were going to be made.'* (White male).

Generation of an allocation sequence was performed by an external statistician and withheld until the end of the trial. This was successful in achieving allocation concealment, along with use of sealed opaque envelopes, as the researcher could not predict what the next treatment allocation was. The researcher collecting outcome data was not blinded to the group allocation, but this would have had little impact on the findings due to the use of objective outcome measures such as laboratory analysis for blood results. The only exception was waist circumference, which although not subjective, was measured by the researcher. It was not appropriate to use 'usual diet' as a control group as this would expose which group participants had been allocated to.

#### **Appropriateness of trial design: stratification**

Participants were stratified according to gender and smoking status. For a future trial, stratification will also include socio-economic status, as discussed in section 2, page 117. Determination of smoking status for stratification proved problematic. Ex-smokers were categorized as non-smokers for stratification during allocation. It transpired later in the study that one individual who had been classified as a non-smoker, smoked shisha once a month (male in Mediterranean Portfolio group). Difficulty also arose with participants who changed smoking status during the trial, or smoked substances other than tobacco. One male subject from Mediterranean Portfolio group restarted smoking during the study. Three males stopped smoking



during the study (2 in Mediterranean Portfolio group), one of whom restarted, then quit again and continued to vape nicotine (Mediterranean Portfolio group).

The other issue was use of illicit drugs, such as crystal methamphetamine (male in low saturated fat group) and injecting mephedrone (2 males, one in each group), as these were disclosed during the study, not at the outset. Participants were asked about use of cocaine at baseline, due to its effect on cardiovascular risk, but not other drugs. Drug misuse was not in the exclusion criteria, but the side effects of these drugs are relevant to the study outcomes, being reported to increase blood pressure.

#### **Appropriateness of trial design: participant views**

At month 6 participants were asked to complete a Process Evaluation questionnaire that included questions regarding their experience during the trial. A summary of the responses provided to the Likert-scale questions is shown in Table 19. To allow direct comparison in Table 19, questions with negative statements were inverted.

Overall, participants were satisfied with various aspects of the trial procedures and design, agreeing or strongly agreeing with the positive statements, and disagreeing or strongly disagreeing with the negative statements. The resources that were used to support the dietary intervention, such as leaflets, recipes and websites, were generally well received. There were differences between the two groups, reflecting the resources that were specific to each intervention. The low-fat recipes given to

Table 19. Process Evaluation responses on participant study experience at month 6.

	Agree or Strongly agree (%)		Didn't use or Don't know (%)	
	Diet1 (n=30)	Diet2 (n=27)	Diet1	Diet2
<b>Leaflets</b>				
Easy to understand	97	100	0	0
Useful	97	100	0	0
<b>Recipes</b>				
Time consuming	48	15	7	0
<b>Websites</b>				
Enjoyed accessing	73	52	17	37
Useful	72	58	21	39
<b>Support</b>				
Good telephone support	68	70	32	26
Good support from dietitian	97	93	0	4
Prefer group than 1:1 consultation	11	16	14	4
<b>Monitoring</b>				
Food diary was difficult	43	18	0	4
Would prefer more visits	30	12	3	8
<b>Maintenance</b>				
Now keep a record of eating	48	52	17	4
Sets nutrition goals for self	82	85	0	4
<b>Randomisation</b>				
Satisfied with group allocated to	80	96	17	0
<b>Experience</b>				
Was enjoyable	90	100	3	0
Shopping and cooking time consuming	43	15	3	0
Easy to find foods in shops	86	89	4	0
<b>Knowledge &amp; understanding</b>				
Helped dispel myths	97	92	0	8

Diet1 participants were found to be time consuming by 48% (another 7% did not use them), whereas the recipes incorporating Portfolio foods were used by all the Diet2 participants, with only 15% finding them time consuming. Websites were found to be useful and enjoyable to more participants in Diet1 (73%) than Diet2 (56%), one third (38%) of whom did not use them. This possibly reflects a reliance on the immediately accessible paper leaflets and recipes for Diet2 who needed to incorporate unusual foods straight away, whereas Diet1 participants were undertaking minimal dietary changes that were more familiar and were more inclined to search for information on the Internet. These findings were confirmed by the responses to an open-ended question seeking the most useful components of the intervention. Leaflets, recipes and websites were named as the most useful components by 10, 8, 7 respondents respectively, and specifically the leaflets on the fat content of cheese, and food labelling (7 responses).

The majority of participants from both groups reported receiving good support from the dietitian, including telephone and one-to-one consultations, and enjoyed the experience of being involved in the trial. This was confirmed by 9 open-ended question responses reporting the interaction with the dietitian as being the most useful aspect of the trial, with reference to discussion around cooking methods, menu options, and her flexibility in arranging appointments and venues. A small number (11% in Diet1 and 16% in Diet2 respectively) indicated a preference for group sessions rather than individual consultations. The number of trial visits for support was deemed adequate, with less than one third of participants indicating a

preference for a greater level of support. Participants reported an improvement in knowledge with the dispelling of previously held myths.

Responses were mixed about the ease and value of food diaries. The majority did not have difficulty completing them (57% Diet1 and 78% Diet2 respectively), with half continuing to use food diaries as a way of monitoring their ongoing intake, and four participants stating that food diaries were the most useful aspect of the trial. Given the time burden of completing food diaries for 3 days, it was considered important to examine participant views further, which are discussed below. Setting nutrition goals is another indicator of the 'maintenance phase' of behaviour change; and was continued by the majority of participants from both groups (82% and 85% respectively). Most participants did not find shopping and cooking to be more time consuming than usual and were able to find the Portfolio foods when shopping.

Open-ended questions were incorporated into the Process Evaluation questionnaire (PE2) to elicit participants' views regarding the strengths and weaknesses of the programme, and their suggestions for improvement. Responses are presented in Table 20 coded in black, green and red fonts, respectively. Comments varied between Diet1 and Diet2 participants, due to the difference between complexities of intervention.

Table 20. Responses from process evaluation questionnaire and semi-structured interviews.

Diet1 Strengths	Diet2 Strengths	Weaknesses	Suggestions
<b>ORGANISATION - TRIAL PROCESS, DELIVERY, MONITORING</b>			
'Was all good' 'Programme is very good' 'Very well organised'	'Like MyNetDiary' (food diary) x2 'Was good it was slow' (introduction of foods) 'Trying foods that I would never buy' 'Being able to try all the new healthier options' 'Food was bought and I was given a chance to test it and see if I liked it in my own home before buying with own money' 'Bag of goodies to give me' 'Flexibility of yourself with appointments, you made every effort'	'Completing lots of questionnaires' 'Food diary' x2 'Stop food diary' 'Expensive foods' x5, 'Stop urine collection'	'Slower phasing in' (of new foods) 'Would prefer UK food diary app' 'Need multiple forms of communication, in case run out of credit' (texts in addition to email and phone) 'Alternative cheap options for food could be possible e.g. all soya products are branded rather than generic' 'Want more flexibility' (Choice of what to change in diet)
<b>SUPPORT</b>			
'So helpful I am really happy with the support and it changed my life' 'Dietician very informative and approachable' 'Great encouragement from Clare & Joan' 'Explanations clear and very helpful information' 'Easy to understand and follow'	'Working with Clare, changing the way I eat and think about food most of the time' 'Focused/treated really well' 'Good support from clinic' 'Talking things through; it helped that I liked you' 'You explained everything to me, so I think I have an understanding of what it was I was trying to achieve'	'Waiting for bloods to be taken' 'Nursing staff in ID outpatients not always quick at ensuring blood appointments are on time'	'Peer support, maybe meet up once or twice as a group to share experiences' 'Share ideas with others' 'Buddy system, but also a forum, where people can share ideas' 'Not comfortable with bake off' 'Website or group like Facebook' 'Talk to others, share cooking, support group, but I don't think probably for me' 'Like the brainwave page of <i>Take a Break</i> mag, how to use your beans'



BEHAVIOUR CHANGE Diet1	Diet2 Strengths	Weaknesses	Suggestions
<p>'Changed my diet and my eating fatty food habit it's all improved'</p> <p><b>KNOWLEDGE</b></p> <p>'Better awareness of the worst foods'</p> <p>'It was interesting to gain knowledge of how different fats effect the heart'</p> <p>'Help with fat versus sugar argument. The whole awareness factor was positive concerning food groups'</p> <p>'I have developed so much interest of eating healthy foods'</p> <p><b>REINFORCEMENT</b></p> <p>'But it is exactly the same diet I was using before this programme'</p> <p>'Confirmed use of healthier choices'</p>	<p>'Very educative and raised my awareness about healthy eating'</p> <p>'Understanding the food labels simply, the benefit to my health'</p> <p>'It has broadened my knowledge of healthy food and gave me choice to select the best suited food for me without compromising the benefits'</p> <p><b>EXERCISE</b></p> <p>'Keeping a healthy steady weight'</p> <p>'Continued swimming'</p> <p>'Started cycling'</p> <p>'Now go to gym together'</p> <p>'Exercise to lose weight'</p>		<p>'Programme should include exercise'</p> <p>'Want to monitor exercise with step counter, MyFitnessPal, NetMyWalk, Fitbit'</p>
EXPERIENCE			
<p>'I found the whole experience enjoyable, interesting'</p> <p>'Have enjoyed it'</p> <p>'It has been a good experience and interesting, enjoyable'</p>	<p>'Enjoyed eating the new healthy foods'</p> <p>'Interesting, enjoyable'</p> <p>'I have enjoyed trying new foods'</p> <p>'I have got more energy'</p> <p>'I have discovered new foods that are healthier and new ways to cook'</p> <p>'Given me a broader scale of adventure to using different foods I don't usually eat'</p> <p>'Japanese food was interesting'</p>	<p>'Having to try new stuff'</p>	<p><b>Key to source of quotes:</b>  PE2 strengths in black font,  PE2 weaknesses of programme in green font,  PE2 suggestions in red font,  Diet2 interviews in blue font</p>

Feedback was also sought from Diet2 participants on the appropriateness of the design of the trial during the semi-structured interviews conducted between month 6 and 12. Questions were asked, 'What changes would you make to the trial?' and 'How could the trial be improved for next time?' with prompts around the trial organisation, appointments, blood tests, urine collection, food diaries, intervention resources, and alternative formats. Quotes are given in blue font in Table 20. The broad themes that emerged were: organisation, support, practicalities, behaviour change, and experience.

Participants were very positive about the design and execution of the trial, describing the whole experience as enjoyable. Unanimous support was vocalized for the provision of foodstuffs during the trial, which were described as a '*bag of goodies*' (White male).

*'But for this research it was good because you're telling someone to change, but you're giving them things to try first. Then if they like it, they'll continue. So sometimes you want to reach the goal, but you can't because of financial constraints. But because it helped that you provided the things, then if the person sees how good it is and how you change, then it's actually good. So, for me, it didn't give me stress on the financial side. (Black African female)*

The professional support was appreciated both from the perspective of increased knowledge and understanding of the diet, and also through a sense of collaboration, with helpful encouragement and approachable manner. One individual referred to the importance of the patient-professional relationship when negotiating behaviour change,



*'It helped that I liked you' (white male).*

One of the most frequently reported strengths of the trial was the practical approach that enabled easy application of shopping, cooking and planning meals. Comments about changed behaviour also featured strongly, whether increased awareness, or specific changes such as checking food labels for fat content. Some participants in the Diet1 group felt that change was not required, as their existing food intake was identical to the dietary recommendations. Many participants interpreted the intervention as an overall lifestyle change and introduced some form of exercise into their routine.

The main criticisms were excessive waiting for clinic nurses to take blood samples, and the cost of foods. Unpopular activities were collection and transportation of 24-hour urine and completing numerous questionnaires.

Suggestions for improvement of the trial design included:

- Intervention to include exercise, with visual monitoring and feedback;
- Resources to include recipes in photo form, weekly meal plans, portion sizes;
- Slower introduction of new foods, with cheap unbranded options;
- To incorporate an element of choice in the trial design, with flexibility in dietary/behaviour changes;
- Peer support with cooking/taste testing;
- Streamlined phlebotomy service;
- Mobile phone to enable arrangement of appointments via text.

### Appropriateness of trial design: measurement of outcomes

Retrieving physical activity data from the accelerometers was problematic due to inadequate wear time, loss of the gadget (it was left on the train), and malfunction after being put in the washing machine. A potential reason for the low priority given to the correct wearing of accelerometers could be that with the device used (the Actigraph), wear data are not visible during collection. This lack of transparency made one participant feel that he was being electronically tagged. Another participant commented that he preferred using his mobile phone to record his step count, as it provided him with immediate feedback. A couple of individuals reported new engagements with exercise, starting cycling at weekends, going to the gym, as the perception was that improving health involved physical activity rather than diet alone.

Overnight fasting was required for accurate assessment of LDL-cholesterol and arterial stiffness. Early morning appointments were not always convenient for participants due to work patterns or taking children to school. Logistically, it was more difficult for the researcher to book appointments with multiple participants on the same day, particularly with the complication of travelling to different recruitment sites.

Measurement of pulse wave velocity was more difficult in obese participants, as the thigh cuff would slide down the leg and fail to maintain pressure.

### Appropriateness of trial design: Participants' experience completing food diaries

Participants were asked about food diaries in the Process Evaluation questionnaire at month 6. Responses were mixed about the ease and value of food diaries. The majority did not have difficulty completing them (57% low saturated fat and 78% Mediterranean Portfolio group respectively), with half continuing to use food diaries as a way of monitoring their ongoing intake, and four participants stating that food diaries were the most useful aspect of the trial.

To examine this issue further, a separate survey was conducted in Mediterranean Portfolio group on their views and experience of using food diaries during the trial. 10 men and 7 women responded, predominantly within the 35 to 49-year-old age category. Sixteen (94%) participants owned an Android or Apple smartphone with access to the electronic food diary (a free app called MyNetDiary), but only 4 (24%) participants chose to use it in preference to a paper food diary, which was selected by 11 participants (65%). Two participants (12%) used both paper and electronic diaries.

Paper diaries were chosen due to ease of use, availability, and preference for writing. The electronic diary was chosen due to convenience, speed, ease of use, including scanner functionality and ability to automatically add regular foods without re-entering data, and user feedback on daily intake of nutrients. Reasons cited for the rejection of MyNetDiary were: limited Internet access, and lack of specific branded foods in the database (from Aldi supermarket). Two participants had previously used

‘MyFitnessPal’, which was preferred due to the easier interface, larger database of UK foods, and integration with daily activity levels. The majority of participants reported that the food diary was easy to use (Figure 21).

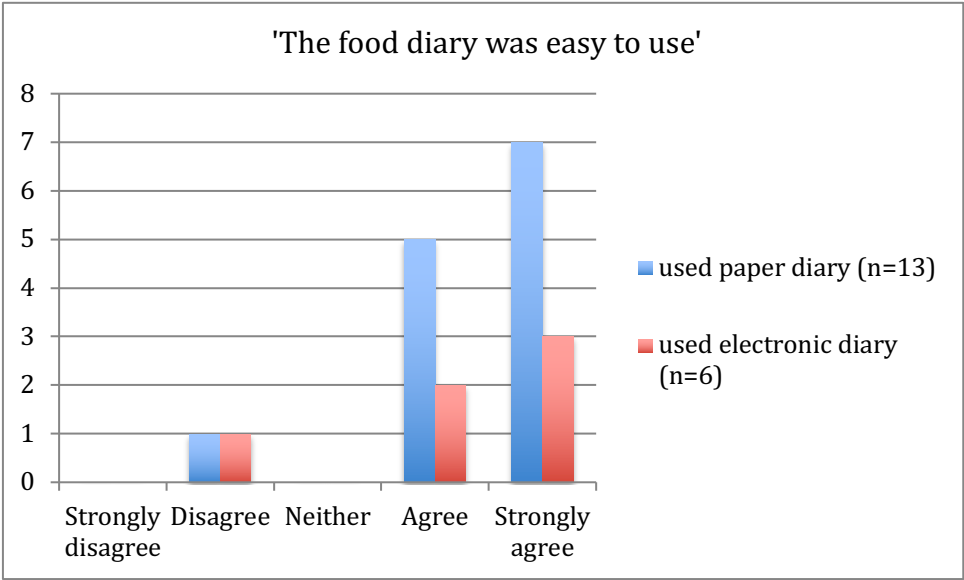


Figure 21. Participant responses to: 'The food diary was easy to use'.

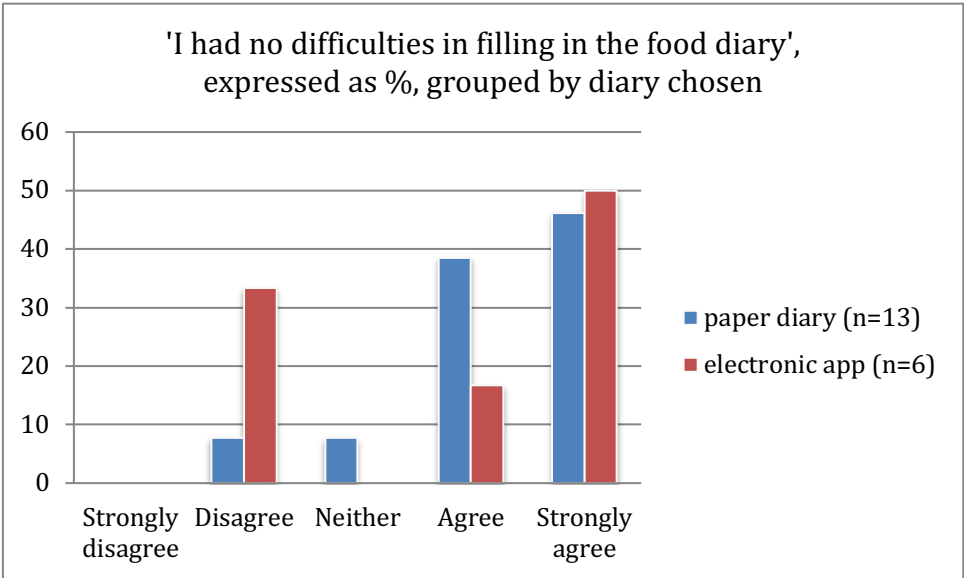


Figure 22. Participant responses to: 'I had no difficulties in filling in the food diary'

When this was rephrased in a separate question as ‘I had no difficulties in filling in the food diary’, the response differed depending on the mode of collection. Participants reported experiencing more difficulties with completion of the electronic app than paper food diaries (

Figure 22).

Participants were asked to select the difficulties encountered during food diary completion from a pre-specified list. When presented with examples of difficulties, participants indicated that estimation of portion size and remembering to document intake were the most common difficulties encountered (

Figure 23). The statements that received no responses were those indicating lack of understanding: ‘I did not know what to do’, ‘I did not understand why I needed to fill it in’, and ‘It was too complicated’.

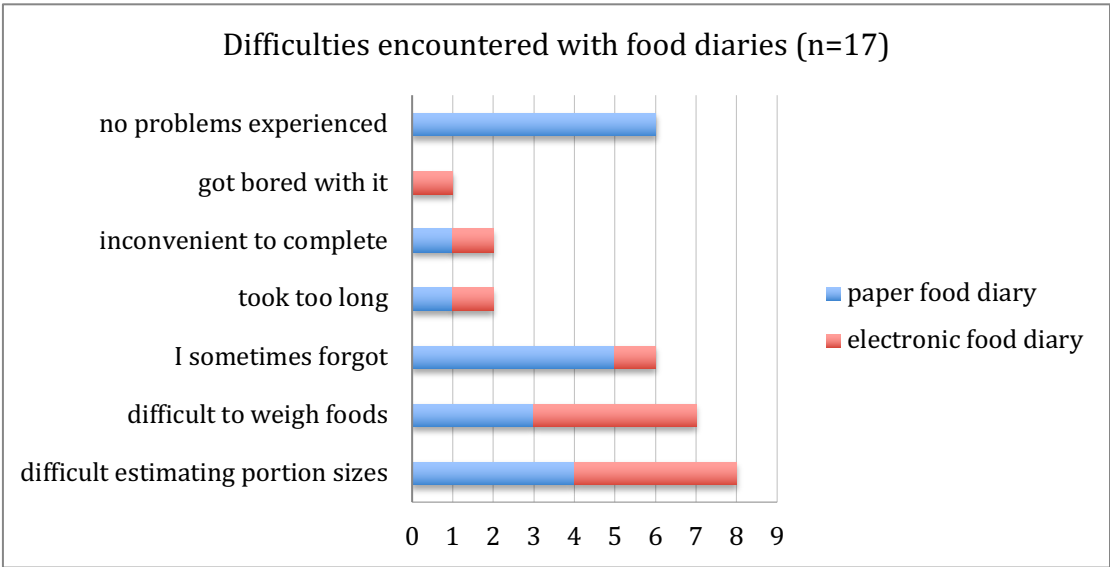


Figure 23. Participant reported difficulties with food diary completion.

Participants reported that they remembered to complete their food diaries, irrespective of which type was chosen (Figure 24). Over half (10/17) said they would continue to use a food diary as a way of monitoring their food intake. Those who used the electronic food diary were more likely to respond positively about the food diary and continue using it as a way of monitoring their food intake.

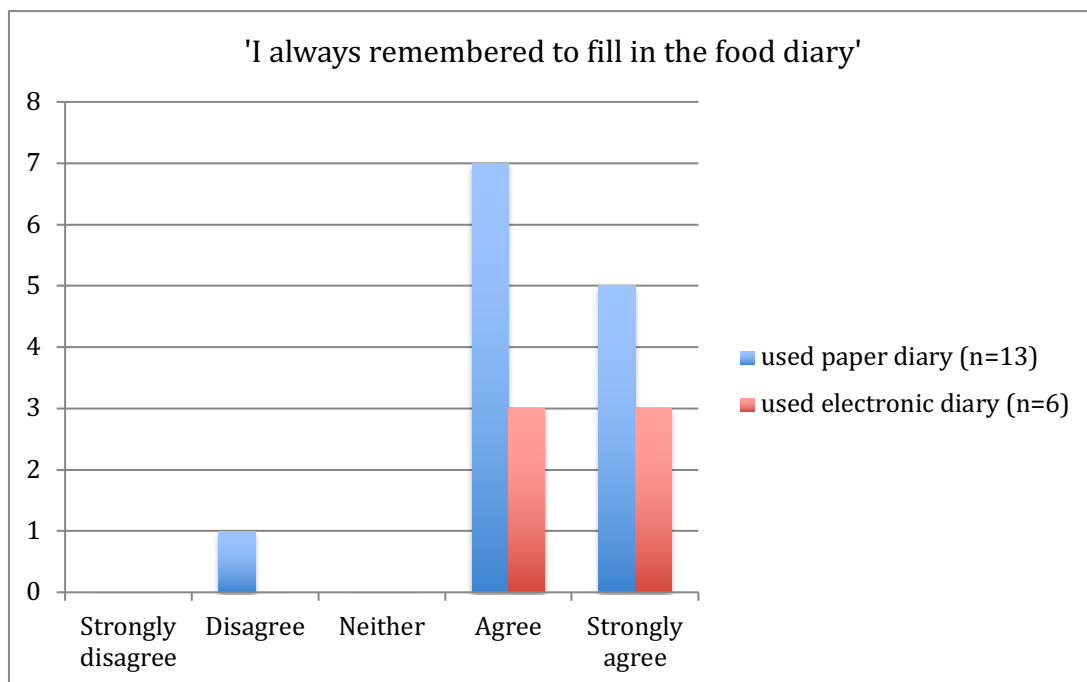


Figure 24. Participant responses to: 'I always remembered to fill in the food diary'

## Feasibility Summary

Trial procedures were found to be acceptable from the participants' perspective and appropriate with regards to systematic screening, randomisation, allocation concealment and stratification, resources used to support dietary intervention,

outcome measurements, and overall organisation. Paper food diaries were selected in preference to the electronic app MyNetDiary. Suggestions on how a future RCT should be conducted were proposed following participant feedback and are included in

Table 21

Table 21. How each finding will prepare for future RCTs

<i>Section</i>	<i>Problem / Specific finding</i>	<i>Solution / Changes to be considered for definitive trial</i>
<b>1.1 Recruitment</b>	Only 16% of cohort was eligible. Eligibility criteria time consuming to apply.	Widen eligibility criteria: remove comorbidities from exclusion list.
	Recruitment slow: Target recruitment rate not achieved; patients declined due to 'too busy'; Unable to contact patients to book appointments; Subjects declined due to specific lifestyle preferences.	More realistic target rate of 10 participants per cohort of 1000. Reduce participant time burden with fewer outcome measurements, fewer attendances, not fasting. Researcher to use mobile phone. Incorporate an element of choice in the trial design, with flexibility in lifestyle changes.
<b>1.2 Attrition</b>	Half of attrition occurred in first month.	Introduce run in period on first stage of diet (low fat diet).
<b>2.2 Outcome measure</b>	LDL-cholesterol and arterial stiffness required overnight fasting; early morning appointments inconvenient.	Avoid measurements that require fasting, e.g. non-HDL cholesterol.
<b>3.1 Fidelity</b>	Issues with food procurement/storage.	Supermarket deliveries of food samples.

	Fidelity of motivational interviewing requires assessment.	Explore use of motivational interviewing treatment integrity code.
<i>Section</i>	<i>Problem / Specific finding</i>	<i>Solution / Changes to be considered for definitive trial</i>
<b>3.1 Fidelity</b>	Motivational interviewing not appropriate for individuals who are ready to change.	Incorporate an element of choice in the trial design, with flexibility in dietary/behaviour changes; match clients to treatment approach.
<b>4.3 Stratification</b>	Potential imbalance between groups for socio-economic status. Changes in smoking status during trial.	Stratify for gender, smoking, recruitment centre, and socioeconomic status. Clarify recreational drug use, including party and chemsex.
<b>4.4 Participant feedback - Organisation</b>	Waiting for phlebotomy. Missed appointments due to lack of phone credit or wifi, not answering 'caller withheld' number.	Streamline phlebotomy service. Mobile phone to enable arrangement of appointments via text. Slower introduction of new foods.
<b>Participant feedback – Support</b>	Liked variety of resources: leaflets, recipes, websites, food labelling, food samples. Continue one-to-one consultations, setting nutrition goals. A minority requested group sessions.	Resources to include photos, portion sizes and meal plan. Peer support with cooking/taste testing, group sessions, online forum, interactive platform for posting top tips for cooking and cost savings.
<b>Monitoring</b>	Food diaries: assisted discussion during consultation and provided model for participants who chose to use for monitoring. Urine collection inconvenient. Actigraph devices disliked, seen as invasive, as data unseen.	Offer UK version of food diary app. Use Mediterranean Diet Score for assessment of dietary adherence as less onerous. Reduce volume of questionnaires. Intervention to include exercise, with visual monitoring and feedback.



## CHAPTER 4: DISCUSSION

### Principal findings: clinical outcomes

We demonstrate for the first time in the HIV population, efficacy of the Mediterranean Portfolio Diet in reducing cardiovascular risk factors. This study shows that dietetic advice to follow a Mediterranean style diet containing nuts, oats, beans, soya protein, and plant stanols produced greater improvements in diet quality (24%), blood pressure (7mmHg reduction) and LDL-cholesterol (10% reduction) than standard guidelines to reduce saturated fat intake in people with HIV infection on antiretroviral treatment.

### Within context of previous studies in this field

#### Portfolio cholesterol-lowering foods

This is the first trial to examine the use of Portfolio foods for the treatment of dyslipidaemia in the HIV population. These novel findings using Portfolio foods within a Mediterranean style diet are important as they provide evidence for a causal link between dietary intervention and LDL-cholesterol reduction within the setting of HIV infection, despite the complex aetiology of the dyslipidaemia, inflammation secondary to viral infection and side effects from antiretroviral medication.

Previous studies have been observational and found no effect of cholesterol-lowering foods on lipid profile in HIV. An uncontrolled before and after study in Brazil, provided

a compound containing 10g soy protein, 20g oat bran and 10g flaxseed alongside guidance for a low-fat diet to 89 adults with dyslipidaemia on antiretroviral treatment. Lipid levels remained stable (LDL-cholesterol 3.2mmol/l,  $P = 0.8$ ) in the analysis of the third of participants ( $n = 31$ ) who regularly used the compound over 3 months (half of whom were taking lipid lowering medication) (Ferreira et al. 2013). Adherence levels or reasons for discontinuation were not reported therefore it is difficult to speculate on the reasons for lack of findings, beyond that of small sample size, confounding due to concomitant use of lipid lowering drugs, and lack of robust methodology (non-randomised design with 58 participants excluded from the analysis due to poor adherence or loss to follow-up).

### **Mediterranean Diet**

Observational studies examining relationships between the Mediterranean Diet and metabolic parameters have also been conducted in the HIV population but failed to find associations with LDL-cholesterol. Favourable associations have been reported with insulin resistance and HDL-cholesterol in patients on ART, but only in those with fat redistribution, such as visceral fat accumulation and/or subcutaneous fat wasting (Tsiodras et al. 2009). Another study found no association between adherence to the Mediterranean Diet and plasma lipid changes during the first year of ART treatment (Turcinov et al. 2009) The lack of findings in observational studies may be due to the method of calculation of high/low adherence to Mediterranean diet. Firstly, dietary intake was assessed at one time point, which may not be an accurate reflection of the fluctuating intake over the 6-year period during which initiation of antiretroviral

therapy and measurement of lipid profiles occurred. Secondly, median consumption was used as the cut-off point in each food category. Therefore, if intake, of say legumes, were low, then using the median would result in a low-level intake being defined as indicating greater adherence to the traditional Mediterranean diet.

Whereas by contrast in the current Best Foods For your heart study, absolute values are used in the 14-item Mediterranean Diet Score to dictate the distinction between high and low adherence. For example, in the current BFF study, only 22% (13/60) of participants were eating the quantity specified by the Mediterranean Diet Score of 3 or more servings per week of legumes at baseline, whereas using the median would define 1.5 servings per week as high adherence.

To date, there has been only one trial, a small pilot in Hong Kong, to examine the Mediterranean Diet in people with HIV infection. A power calculation was not reported, but they chose to test for differences and reported a detrimental 0.52mmol/l increase in total cholesterol in the Mediterranean diet group ( $P = 0.01$ ) and no significant difference between Mediterranean and low-fat diet groups ( $P = 0.12$ ) (Ng et al. 2011). It is likely that the sample size of 48 was inadequate to detect an effect size, should it exist, and the study had other limitations. Firstly, the broad eligibility criteria resulted in the inclusion of unstable patients who influenced the high attrition rate of 25% (12/48), with 4 deaths during the study from AIDS related complications. These unstable patients included those newly diagnosed with HIV, recently commenced on antiretroviral therapy, and changing antiretroviral regimens during the study, all of which would impact blood lipid levels independently of dietary intake.

Secondly, examination of the nutritional content showed that the dietary interventions of the two groups appeared to differ in delivery rather than content. Due to the Mediterranean diet being very different to the local Chinese diet, the researchers decided that only small changes would be acceptable, and therefore advised participants to include 1 serving per day of 3 items of their choice from a list. These included: white meat to replace red meat, use of rapeseed or olive oil in cooking, beans/tofu to replace meat, nuts, low fat dairy/soya drink instead of the full fat version, 5 servings of fruit and vegetables. The intervention was therefore not truly reflective of the Mediterranean diet. This study also differed from ours with respect to the population; their study included a higher proportion of men (77%), all of Chinese or Asian origin, 21% not on antiretroviral medication, lower total cholesterol levels (mean 4.7mmol/l), and with an unknown number who were taking lipid-lowering agents.

Whilst not explicitly named a Mediterranean diet, the intervention in a Spanish RCT had Mediterranean tendencies, with advice emphasising the benefits of eating oily fish, olive oil, vegetables and fruit (Saumoy et al. 2016). Thus, it would meet the inclusion criteria for the Cochrane meta-analysis on Mediterranean dietary patterns for the prevention of CVD (Rees et al. 2012). The intervention also included aerobic exercise three times a week and support to quit smoking, whilst the control group received information sheets; both were followed-up every 4 months for 3 years. A significant difference between groups in LDL-cholesterol was reported at 24 months ( $P=0.01$ ), but not at 36 months, which was the primary outcome (actual figures not

reported). A power calculation was not performed; therefore, it is unclear whether the sample size of 54 men was adequate to detect a difference in any of the outcome measures of lipid levels, CVR and carotid intima-media thickness (Saumoy et al. 2016). Comparisons with the current Best Foods For your heart study are hampered by the difference between interventions, lack of assessment of dietary intake, all male population with high CVR (median Framingham score 16%), high level of subclinical atherosclerosis at baseline (83% displayed presence of a plaque or carotid intima-media thickness >1.5mm during ultrasound imaging of the carotid arteries), and wide use of statins (46% at baseline and a further 10 in each group commenced on statins during the study).

#### Other cholesterol-lowering dietary interventions

Other dietary RCTs conducted in the HIV population (Belloso et al. 2006; Fitch et al. 2006; Balasubramanyam et al. 2011; Fitch et al. 2012) have utilised an intervention that includes exercise and is aligned with the National Cholesterol Education Programme (NCEP ATPIII expert panel 2002), focusing on reduction of saturated fat to <7% of energy. Despite the mention of increased fish and fibre in some studies, this intervention is closer to the advice for our low saturated fat group, than the Mediterranean Diet. Even with improvement in blood pressure, waist circumference (Fitch et al. 2006), inflammatory markers (Fitch et al. 2012), and HDL-cholesterol (Belloso et al. 2006), all of the trials failed to find a change in LDL-cholesterol. The pooled estimate for these studies was reported in a systematic review (Stradling et al. 2012) showing no significant differences between dietary intervention and control

groups for LDL-cholesterol  $-0.01\text{mmol/l}$  (95%CI  $-0.81$  to  $0.79\text{mmol/l}$ ,  $P = 0.98$ ). This is consistent with findings from the healthy adult population, such as the FIT-Heart study (Mosca et al. 2008) which showed no effect with NCEP (no difference in mean percent change in LDL-cholesterol between groups at 1 year,  $P = 0.64$ ), and subsequent debates have questioned the low levels of fat intake recommended by NCEP and suggested the need to test modifications of the Mediterranean diet instead (Mitka 2009).

The only NCEP diet study in HIV to report a mean difference in LDL-cholesterol between groups at 6 months ( $-0.38\text{mmol/l}$ , 95%CI  $-0.66$  to  $-0.1$  [my calculation],  $P=0.009$ ), was a Thai trial that randomised 72 adults with LDL-cholesterol  $>2.5\text{mmol/l}$  (mean LDL-cholesterol  $4\text{mmol/l}$ ). They reported a significant reduction in LDL-cholesterol ( $-0.56 \pm 0.1\text{mmol/l}$ ,  $P<0.0001$ ) for the participants in the intervention group, but only analysed the 29 who had lipid results, therefore it was not intention-to-treat analysis. The study was also only powered to 50% to detect  $1\text{mmol/l}$  change in LDL-cholesterol at 24 weeks, raising questions about the validity of the  $P$  values, that were not substantiated by reporting 95% confidence intervals (Almeida et al. 2011). Other sources of bias include attrition of 18% and failure to describe the allocation generation and concealment.

Two other studies, that followed NCEP principles, took a slightly different perspective in that they examined dietary intervention as prevention for metabolic abnormalities, excluding subjects with raised lipids. The interventions included some components of

the Mediterranean diet such as advice to increase wholegrains, fruit and vegetables. The first Brazilian study found no significant difference between groups at month 12, but trends toward reduction in LDL-cholesterol ( $-0.18\text{mmol/l}$  intervention group  $P = 0.047$ ) and increase in fibre intake ( $+12\text{g}$  intervention group and  $+9\text{g}$  control,  $P < 0.001$ ) (Lazzaretti et al. 2012). The study was beset with limitations including small sample size of 53, multiplicity comparing outcomes between groups and at 4 different time points, unclear allocation generation and concealment, and most importantly, recruitment/clinical bias as participants had been receiving antiretroviral medication for median of 8 months at baseline, therefore it was not a prevention study as lipid disturbances were already likely to have occurred. In contrast, a different Brazilian research group demonstrated ingenuity by recruiting 83 participants at the point of antiretroviral therapy initiation before randomizing for one-off dietary intervention (control) or intervention with ongoing dietetic support (Webel et al. 2018). After 1 year, 21% of the intervention group had developed dyslipidaemia, compared to 68% in the control ( $P < 0.001$ ). Attrition was low and crude mean difference between groups in LDL-cholesterol was  $0.6\text{mmol/l}$  ( $P = 0.006$ ). A third study that also advised participants to increase wholegrains, fruit and vegetables, but within a weekly group setting that included advice on exercise, described weight reduction  $-0.7 \pm 3.7\text{kg}$  at month 3 in the intervention group compared with a gain of  $0.2 \pm 3.4\text{kg}$  in the control group ( $P = 0.03$ ). There was no improvement in the primary outcome measures of weekly moderately vigorous physical activity or Healthy Eating Index, and lipids were not measured in this group of 107 people living with HIV in the Cleveland community of America (Dinu et al. 2017).

## Summary

In contrast to our findings of LDL-cholesterol reduction with the Mediterranean Portfolio diet, meta-analyses on the Mediterranean Diet have found no association including a recent umbrella review of 6 studies that indicated no evidence of a link between greater adherence to Mediterranean diet and LDL-cholesterol in the general population (MD -0.11, 95%CI -0.24 to 0.02) (Jenkins et al. 2006). The lack of cholesterol reduction with the Mediterranean diet would suggest that the lipid lowering effect is due to the inclusion of specific cholesterol-lowering foods such as plant stanols, soya protein and beta-glucan. Our findings are consistent with the literature in non-HIV population where Portfolio foods reduced lipid levels by 0.6mmol/l at 1 year (Estruch et al. 2018), as previously discussed in the introduction.

One of the studies included in Dinu's meta-analysis was PREDIMED, the first Mediterranean diet RCT to use clinical endpoints in primary CVD prevention. Unfortunately, firm conclusions on the effect of Mediterranean diet on all-cause mortality cannot be drawn due to methodological concerns regarding unclear allocation concealment. However, this large study suggested a strong protective effect of the Mediterranean diet against CVD with favourable effects on each outcome with point estimates in a beneficial direction (Silverman et al. 2016).



## Possible mechanisms

Our findings support the hypothesis that dietary intervention can significantly reduce LDL-cholesterol in people with HIV infection despite the additional burden of inflammation. Whilst the underlying mechanism of action specific to HIV infection remains unknown, there is no reason to believe that it would be any different to that previously proposed in the general population for non-statin therapy (including diet), via up-regulation of LDL-cholesterol receptor expression (Goldstein & Brown 1987). Thus, the low intake of saturated fat deprives hepatocytes of cholesterol, stimulating LDL-cholesterol receptor production, which reduces LDL-cholesterol levels by removing LDL-cholesterol from blood by receptor-mediated endocytosis (De Smet et al. 2012). The mechanism for cholesterol reduction from Portfolio foods is different. Taking the example of plant stanols, there are two paradigms currently favoured for explaining their LDL-cholesterol lowering activity: reduction of intestinal cholesterol absorption, and stimulation of transintestinal cholesterol excretion, but the exact molecular mechanisms is likely a complex interplay of multiple processes (Berger et al. 2004). Understanding the mechanism is important, as using plant stanols to decrease the absorption of cholesterol could impact the uptake of fat-soluble vitamins. The evidence for the impact of plant stanols on fat-soluble vitamin status is largely inconsistent, with a similar number of studies reporting reduced versus no effect on blood levels of carotenoids, tocopherol, and lycopene (Baumgartner et al. 2017). In the current study, the significant reduction in vitamin E levels at month 6 (-4.6umol/l, 95%CI -7.5 to -1.7,  $P<0.01$ ) and month 12 (-3.7umol/l, 95%CI -6.8 to -0.6,

P = 0.02) were no longer evident when levels were standardised for levels of total cholesterol (P = 0.1 for both). The reduction observed in non-standardised concentrations is likely to be due to reduced carrier capacity, as tocopherols are transported by lipoproteins. This pattern has been reported in a meta-analysis of 41 RCTs, after accounting for the fall in cholesterol levels, levels of vitamin E were not changed after plant stanol consumption, although  $\beta$ -carotene concentrations remained significantly depressed (-10.1%, 95%CI -12.3 to -8.0) (Noakes et al. 2002). This was not the case in the current study where no difference was observed between groups in vitamin A levels, before or after adjustment for cholesterol levels. This finding supports previous work showing that advice to increase the consumption of carotenoid rich fruit and vegetables maintained plasma carotenoid concentrations during 3-week consumption of plant stanols (Schwingshackl & Hoffmann 2014), and provides evidence for the longer duration of 12 months. The unexplained and contradictory cases of vitamin A and E deficiency and excess observed in the Mediterranean Portfolio group (one case of each) are unlikely to be due to the daily intake of plant stanols.

Other potential mechanisms have been postulated for the cardioprotective effect of the Mediterranean diet such as antioxidant or anti-inflammatory effects (Vos et al. 2016). Inflammation continues despite successful treatment of HIV and contributes to the progression of atherosclerosis, as illustrated by the clear association between inflammatory markers (specifically hs-CRP, D-dimer and IL-6) and CVD in people with HIV infection following a review of 33 datasets and 48 immune markers of

inflammation (Višković et al. 2018). Similar inflammatory biomarkers (hs-CRP, IL-6, CD40L, tPA, MCP-1, IL-8, and P-selectin) have been found to be associated with HIV dyslipidaemia, such that a one-unit increase (mmol/l) of total cholesterol was associated with a 1.41 fold (95%CI 1.13 to 1.76) increased odds of having a greater inflammatory burden score (Schwingshackl & Hoffmann 2014). In the current study the general inflammatory marker, high-sensitivity C-reactive protein, remained elevated and appeared unaltered following the dietary intervention, potentially with a slight trend towards a reduction (-1.35mg/L (95%CI -4.04 to 1.34). This is consistent with evidence from a meta-analysis of 14 RCTs (n = 1,942) demonstrating an association between a Mediterranean dietary pattern and improvement in multiple markers of inflammation and endothelial function (including hs-CRP WMD -0.98mg/l, 95%CI -1.48 to -0.49, I<sup>2</sup>=91%) in adults at risk of CVD (Schwingshackl & Hoffmann 2014). Although the findings are cautionary due to high heterogeneity, a common problem when pooling dietary intervention studies with variation in types of diets used. In this case the criteria qualifying for Mediterranean diet status was broad, being two or more items from a list of Mediterranean dietary components.

Clinical micronutrient supplementation trials have largely failed to show an effect on risk of CVD despite positive findings from in vitro studies verifying the oxidation hypothesis that antioxidants inhibit the development of atherosclerosis by preventing oxidation of LDL-cholesterol in the sub-endothelial space of arteries (Gotto 2003). Epidemiological evidence from cohort studies has also suggested an association between increased intake of specific micronutrients (such as folate and vitamin E)

and CVD (Woodside et al. 2005). However, this has not borne out in clinical trials, meta-analysis of which found no evidence to support the use of antioxidant supplements for the primary prevention of CVD (RR 1.0, 95%CI 0.98 to 1.02,  $I^2=42\%$ ) (Myung et al. 2013). One explanation could be that synthetic supplements are not equivalent to the intake of foods containing micronutrients, such as fruit and vegetables, which contain other nutrients such as phytochemicals. Therefore the potential synergy among nutrient rich foods in Mediterranean diet that promote favourable changes in intermediate pathways cannot be fully dismissed (Goszcz et al. 2015).

### **Strengths and weaknesses of this study**

The strengths of our study include the randomised design, the substantial representation of women and ethnic minorities, and the 88% 1-year follow-up rate.

The main strength of this study lies in its randomised design with intention to treat analysis. An unpredictable allocation sequence was generated externally and concealed from the researcher enrolling participants until assignment occurred, thereby eliminating selection bias at trial entry. Stratification of randomization for gender and smoking status enabled the variance of groups to be matched for those characteristics. The imbalance observed between groups in socio-economic status, most likely resulted by chance, due the relatively small sample size. Outcome measurements were objective and standardised, producing parametric data that were adjusted for the baseline variable, all of which enhance the power of the study.

Despite being a pilot, this study was powered a-priori to detect a clinically meaningful difference of 10% or 0.5mmol/l in LDL-cholesterol between groups. The findings therefore indicate the existence of a causal relationship between the Mediterranean Portfolio diet and LDL-cholesterol in this setting.

Participants were free living, yet attained relatively high levels of adherence to the dietary intervention, compared to that usually observed in trials, such as 60% seen in 27 weight loss studies (Lemstra et al. 2016). This trial was multicentre with broad eligibility criteria, resulting in the inclusion of a wide variety of participants from different ethnicities and a large proportion of women, thereby giving good external validity and greater clinical applicability.

Lack of blinding is an issue with dietary intervention trials as it is virtually impossible for participants to be unaware of their intervention. In this study the researcher was not blinded as she administered the intervention to both groups and was aware of the group allocation during collection of outcome data. Whilst the majority of outcomes were objectively measured, for example blood sample going to laboratory for analysis of LDL-cholesterol, one was more vulnerable to bias (waist circumference). Steps were taken to avoid potential bias by measuring the follow-up waist circumference without reference to the previous value.

Limitations to this study include the possibility of Type 2 error due to inclusion of participants with mild dyslipidaemia limiting the capacity to detect changes in lipids. The -0.5mmol/l reduction in LDL-cholesterol in the fully adjusted analysis is clinically

relevant, however, the lower confidence interval indicates that the effect size could be as small as -0.2mmol/l. This large point estimate with wide confidence intervals suggests that conducting a future trial with a larger sample size, to produce narrower confidence intervals, would be of benefit to confirm the findings. Caution should be exercised with the interpretation of other outcome measures, such as blood pressure, due to potential bias with multiplicity of analyses. Although the study was powered for the primary outcome of LDL-cholesterol, it was not powered to detect numerous outcome measures and therefore some statistically significant findings are possibly the result of chance alone.

Use of a surrogate marker, such as LDL-cholesterol, as the primary outcome would be criticised because it assumes that the cholesterol lowering effects of the diet will slow progression of atherosclerosis and reduce CVE (Ramsden et al. 2016).

Skepticism regarding the causal nature of the relationship between LDL and CVE has existed due to the paucity of dietary intervention RCTs with clinical endpoints. This is unsurprising given the large number of participants required for RCTs and the practicalities of sustaining adherence to a specific dietary pattern over several years. Many trials, including non-statin, support the theory that reduction of LDL-cholesterol will produce a corresponding reduction in CVE (Baigent et al. 2005; Silverman et al. 2016). One such trial, IMPROVE-IT, where the addition of a non-statin lipid-modifying drug to statin therapy showed additional benefit suggesting that all reductions in LDL-cholesterol levels are of benefit, regardless of mechanism (Jarcho & Keaney 2015). There is also emerging evidence that products of lipid metabolism are mediators on

pathway between diet and CVD (Toledo et al. 2017). The controversy has prompted the publication of a Consensus Statement on LDL causality from the European Atherosclerosis Society with presentation of Grade 1 evidence that satisfies all the criteria for causality that LDL-cholesterol is directly implicated in the initiation and progression of CVD, and that reduction of LDL-cholesterol reduces the risk of CVD proportional to the absolute reduction in LDL-cholesterol (Ference et al. 2017).

### Implications for clinicians

The findings from this study provide population specific evidence to support the recommendations made in current HIV guidelines and suggest a potential direction for more explicit guidance on the type of diet, similar to that advocated in America.

National Lipid Association Expert Panel recommend the use of any dietary pattern that has an emphasis on plant foods and lean sources of protein, with nutritional counselling by a registered dietitian to individualise cardioprotective dietary pattern based on the patient's specific dyslipidaemia (Jacobson et al. 2015).

National and European HIV guidelines currently recommend that modifiable risk factors be addressed, including reduction of LDL-cholesterol to reduce CVD risk (Churchill et al. 2016; Ryom, Boesecke, et al. 2018). The lack of trial evidence in HIV has led to use of evidence from the general population, which focused on reduction of saturated fat to improve LDL-cholesterol and reduce CVD risk (Ryom, Boesecke, et al. 2018). The findings from the current trial provide Rating I quality of evidence

(data from one or more RCTs with clinical outcomes and/or validated laboratory endpoints) (Department of Health and Human Services 2018) that may help realign recommendations towards the use of the Mediterranean Diet with Portfolio cholesterol-lowering foods, rather than solely focusing on reduction of saturated fat. However, despite providing evidence of a clinically and statistically significant reduction in LDL-cholesterol within this setting that would lead to a reduction in mortality, strong recommendations cannot be made due to the lack of definitive clinical endpoints. Ultimately this study has the potential to influence future policy, pending confirmation of findings from similar future studies.

### Unanswered questions

This explanatory trial demonstrated efficacy, as the outcome (LDL-cholesterol) was found to be attributable to the intervention (Mediterranean Portfolio diet). The focus on participants not on lipid lowering medication allowed us to demonstrate a clear effect of the Mediterranean Portfolio diet on the lipid profile, however it left unanswered questions about external validity. Although this diet was well received by participants, without the costly provision of sample foods it may be a greater challenge to apply in real life. Future trials are required that are explicitly pragmatic to test effectiveness and answer the question of whether the Mediterranean Portfolio diet will improve patient-important outcomes when applied by typical clinicians to typical patients. Ideally such trials should include clinical endpoints, but this is unrealistic given the financial and time constraints of following a young cohort to



ascertain mortality data. Alternatives would be the interrogation of prospective cohorts, such as Data Collection on Adverse Events of Anti-HIV Drugs study (D:A:D), to decipher the influence of diet on clinical endpoints such as mortality and CVE, however dietary data is not at present collected by any of the large HIV cohorts (Available at: <https://cfar.globalhealth.harvard.edu/pages/research-cohorts> Accessed [1/8/18]).

One such question regarding generalisability is that of long-term adherence to the Mediterranean diet. The regression towards the mean observed at month 12 is consistent with most behaviour change studies, such that cessation of intensive monitoring and support results in loss of effect. Future work could explore whether alternative support mechanisms can mitigate this, such as use of social support systems that has produced higher adherence rates (Lemstra et al. 2016) and increased effectiveness (Greaves et al. 2011) for weight loss interventions. Another avenue could be via virtual support, as recent work has shown that internet-based dietary interventions are modestly effective, with 0.5-point increase in Mediterranean Diet Score (Livingstone et al. 2016). Thus, the challenge remains to produce larger and sustained dietary changes that are also cost effective.

Another issue around generalisability is to acknowledge that the 'one size fits all' approach is not appropriate, as it has produced limited effect on population level disease. For this reason, the current study utilised individualised dietary advice within the framework of well-defined behaviour change techniques, which has been shown

to increase effectiveness of interventions to promote change in diet and physical activity (Greaves et al. 2011).

Diet is only one of many risk factors for CVD. The INTERHEART case control study elucidated the influence of diet on disease burden with lack of daily consumption of fruit and vegetables producing a population attributable risk for MI of 13.7%, comparable to 12.2% for lack of physical activity (Yusuf et al. 2004). Similarly interventions for the Mediterranean diet, yoga or walking displayed comparative effectiveness of CV risk reduction when intervention effect sizes were compared from various meta-analyses of RCTs (Chu et al. 2016). Given the importance of physical activity in CVD prevention, feedback received from BFF participants (see Table 20 and p187), and the lack of effect observed in the current study on metabolic syndrome components such as weight, waist circumference, triglyceride, and glucose levels (Table 12), justify consideration of inclusion of an exercise component within the intervention for future trials. Furthermore, recent research interest in frailty and its morbidity consequences has revealed strong correlations between sarcopenia, central obesity and frailty in people with HIV infection (Hawkins 2018). This apparent contradiction between weight loss and gain elicits questions regarding the potential role of exercise to preserve muscle mass and reverse frailty as exercise may provide beneficial effects on multiple outcomes. Incorporating physical activity into the intervention would be also logical, as studies have shown that structured exercise reduces abdominal obesity in people with HIV (Lake et al. 2017). Meanwhile, evidence from the general population indicates that weight reduction is required to

reverse metabolic syndrome and diabetes (Ferland & Eckel 2011; Lean et al. 2018), and that interventions to promote weight loss are more effective when both diet and physical activity are targeted (Greaves et al. 2011). Given the reduced activity levels (Vancampfort et al. 2018) and increasing prevalence of obesity within the HIV population (Koethe et al. 2016), with its associated implications of increased risk of CVD, cancer and all-cause mortality (Achhra et al. 2018), incorporation of physical activity interventions alongside dietary in future trials would be timely. Mean activity levels in the current study (6,000 steps/day) were comparable to that seen in other studies (5,899 steps/day, 95%CI 5,678 to 6,418, n=252 from 4 studies) (Vancampfort et al. 2018), therefore future studies could include a target such as the public health recommendation of 150 minutes/week of moderate-intensity physical activity, or the popular alternative of 10,000 steps per day (Wattanapisit & Thanamee 2017). This physical activity component could be incorporated into the future trial in a number of different ways. Firstly, assuming that physical activity would be beneficial and increase the effect size of each intervention, it could be added to both arms Diet1 and Diet2. However, disentangling which component provides benefit could prove difficult where the comparison is Diet1+PA versus Diet2+PA. Alternatively, physical activity could be added to both arms but be the sole intervention in the control arm, providing a comparison of PA versus Diet2+PA. This includes a 'true' control for the dietary intervention, reducing risk of contamination between 2 different diets. Another option would be to use a 2x2 factorial trial design so as to assess the effectiveness of each component, with comparison of usual diet / Diet2 / usual activity / PA. However,

this presents the original problem of allocation of some participants to the no intervention quadrant, and whether this would impact recruitment.

In this trial, levels of physical activity were monitored using an accelerometer device. The participant response to wearing a non-interactive device was not wholly positive. With the recent advances in technology, wider availability and lower cost of home use fitness trackers, future trials could use a user visible device such as Fitbit, step counter app on the participants phone, or an app such as MyFitnessPal to simplify and combine monitoring of food intake and physical activity levels. Wearable activity monitors also provide objective, real-time feedback, and have been shown to be cost effective (Abu-Omar et al. 2017), with positive effects on weight loss (MD -1.65kg, 95%CI -3.03 to -0.28,  $I^2=81\%$ , 11 RCTs) and increased physical activity (SMD 0.26, 95%CI 0.04 to 0.49,  $I^2=65\%$ , 12 RCTs) (Goode et al. 2017). Frequency of viewing one's own data has been associated with moderately vigorous activity levels, compared to no effect using a website or app (Hartman et al. 2018).

In summary, unanswered questions highlight the need for pragmatic trials in a wider population (including comorbidities and associated medication), with physical activity and enhanced support strategies to demonstrate effectiveness and sustained dietary change in the long term.

## Conclusion

Mediterranean Portfolio diet is a worthwhile intervention in HIV dyslipidaemia. Following the projection from a systematic review examining mortality endpoints in relation to non-statin interventions, the 0.5mmol/l LDL-cholesterol reduction demonstrated in this study has potential to reduce mortality by 10% (extrapolated from (Silverman et al. 2016)). Dietary intervention needs to address underlying drivers of chronic inflammation in HIV population, not just traditional causality of arteriosclerosis and build-up of cholesterol plaques.

## Principal findings: feasibility aspects of trial

The findings from this trial highlight specific recommendations for future trials, as detailed in

Table 21. Space limitations permit one aspect, intervention adherence, to be discussed in detail here.

### Monitoring adherence to a complex intervention

Quantifying the delivery and uptake of the intervention is one of the most difficult parts of a clinical trial involving a dietary and/or complex intervention. Monitoring food intake is traditionally performed using the food diary to assess nutrient intake. However, for this trial it was important to examine the dietary patterns of participants due to the potential interaction of individual dietary components and their impact on disease being greater than the sum of individual nutrients. The two different approaches to examining dietary patterns ('a priori' and 'a posteriori') have previously been discussed in detail in a review by the author (Stradling et al. 2014). Of the many 'a priori' dietary indices that have been developed to assess adherence to particular dietary patterns, the Mediterranean Diet Score is the most widely studied (Trichopoulou et al. 2014) and was selected for use in this study.

## Development of Mediterranean Diet Score

The first operational score that appeared in the literature was constructed on the basis of the relationship between the mortality of older inhabitants of several rural Greek villages and their traditional Mediterranean diet (Trichopoulou, Kouris-Blazos, Wahlqvist, et al. 1995). This 9-point Mediterranean Diet Adherence Screener has subsequently undergone several developments, being validated (Martinez-Gonzalez et al. 2004), extended to 14 items (Schröder et al. 2011; Martínez-González et al. 2012), and re-validated for the PREDIMED study (Papadaki et al. 2018). It has also been evaluated in the UK population for concurrent validity and re-test reliability using 3-day food diaries (Trichopoulou et al. 2005). When selecting an 'a priori' index to evaluate adherence to the Mediterranean diet from the numerous variations available, this version was chosen for several reasons: it was validated, easy to administer, had been used in non-Mediterranean populations (Stradling et al. 2014), and largely reflected the quantitative and qualitative characteristics of the true early Mediterranean diet pattern of the 1960's.

## Strengths and limitations to Mediterranean Diet Score

Diet indices are useful to measure the extent of adherence to a particular dietary pattern, but they are not necessarily good predictors of morbidity or mortality, as their construction requires arbitrary choices concerning selection of components, their cut-off values, and method of scoring (Zaragoza-Martí et al. 2018). Each issue regarding the construction of a diet quality score will be discussed in turn. With the existence of

numerous versions of scores, comparison and pooling of studies is difficult, and quality is variable as few scores fulfill psychometric properties and applicability parameters expected of scales/indices (Hoffman & Gerber 2012).

### *1) Selection of components - Qualitative characteristics*

The first Mediterranean Diet Score was constructed as a composite scale of food items considered characteristic of the traditional Mediterranean diet. This version of the score has followed the original definition of the Mediterranean diet.

Whilst some qualitative characteristics of the Mediterranean diet are difficult to quantify, such as cooking styles (with use of stone ground sourdough bread), and growing conditions (vegetables in direct ultraviolet light rather than under glass to favour phytochemical production (D'Alessandro & De Pergola 2015)), this score appears to address the majority of distinctions, such as distinguishing olive oil from monounsaturated fat intake and categorising wine as a separate entity to total alcohol (Sotos-Prieto et al. 2015). It is different from other versions of Mediterranean Diet Score, as it specifically addresses the normative or absolute cut-off points for the consumption of food items typical of the Mediterranean diet and inquiries about the consumption of foods that do not fit the traditional Mediterranean diet, such as sugary soft drinks and pastries. This score does not falsely grade cereals favourably, or include potatoes with vegetables, but it does fail to specify wholegrains. Opinions differ as to whether the Mediterranean Diet Score can evolve and be re-purposed using cultural variations, for example questioning the incorporation of Japanese



staples such as soya, seaweed and mushrooms into the 'Japo-Mediterranean' diet as it is not faithful to the traditional diet of the original studies, or the use of alternative unsaturated cooking oils (such as rapeseed, sunflower, and flaxseed), or 'low-fat Mediterranean' diet because the important factor is not the amount, but the type of fat containing phenolic compounds. Others believe that the existing definitions of the Mediterranean diet are not static but can be modernized; for example to exclude wheat bread due to the introduction of new wheat hybrids resulting in an increased prevalence of gluten sensitivity, or expanded, to define a lifestyle with the addition of 7 habits (e.g. drinking wine with meals, and water in between), and 6 activity aspects (e.g. physical activity, taking a siesta, socialising) to the existing 15 dietary components (Trichopoulou et al. 2014), or substantiated, by amalgamating with current scientific evidence of health outcomes associated with individual components (D'Alessandro & De Pergola 2015). For the latter, a literature based 9-item score has been proposed based on weighted medians for consumption of all the food groups in 24 cohort studies (Martínez-González et al. 2017). However, it fails to acknowledge the contribution of nuts and tomato-based sauce (named 'sofrito' in Spain, and 'lathera' in Greece), and does not include negative scoring for butter, biscuits and high sugar items.

## *2) Cut-off values - quantitative*

A large number of 'a priori' indices use the median intake to determine the cut-off of a high/low intake, or adherence/non-adherence to the dietary pattern. Using medians is consistent with the use of food frequency questionnaires to assess dietary intake, as

these tools are better suited to rank individuals rather than to accurately measure absolute intakes (Waijers et al. 2007). However, the cut-offs are then dependent on the sample characteristics and limit generalisability to other populations. Similarly, the median value does not necessarily reflect a level of intake of foods that is consistent with a positive or negative effect on health (Bamia et al. 2017). One study illustrated the falsely optimistic representation of the proportion of participants adherent to the Mediterranean diet as determined by a median cut off (showing 89% participants achieving adherence to  $\geq 1$  daily servings vegetables, and 52% adherence to  $\geq 1.6$  servings fruit and nuts), compared to predefined cut-offs (showing 23% participants achieving adherence to  $\geq 2$  daily servings vegetables, and 13% adherence to  $\geq 3$  fruit, 13%, to  $\geq 0.4$  daily serving nuts) (D'Alessandro & De Pergola 2015). Conversely, the current study illustrates an important loss of information with the use of binary categorisation (D'Alessandro & De Pergola 2015). Approximately one quarter of participants increased their daily consumption of fruit and vegetables in terms of number of servings, but no difference was observed with the yes/no to  $>400\text{g}$  vegetables or  $>2$  fruit as defined by the Mediterranean Diet Score categories, as only 4 participants in each group (13%) changed categories upwards. Therefore, whilst it seemed more appropriate to use a score that utilised the cut-offs of the Greek population rather than taking the group median as a cut-off value, discrimination between individuals might be compromised. Where intake for a certain food remains below the desired cut-off level for almost all subjects in a group, such as red wine in this study, that particular index item will not contribute extra discriminating power. A preferred method would be to let the score for each item

illustrate proportional adherence, such as using a larger number of cut-off points to improve the diagnostic capacity of the score (Trichopoulou, Kouris-Blazos, Vassilakou, et al. 1995).

### *3) Scoring*

One of the debates during the selection of components for an index is how to handle items that are considered both beneficial and detrimental. For example, moderate intake of red wine during meals is one of the unique elements of the Mediterranean dietary pattern, compared to other 'healthy' diets. However, both insufficient and excessive intakes of alcohol are detrimental; therefore, it is critical that wine is distinguished from beer and spirits, as in this score. The original description of the traditional Mediterranean diet (Waijers et al. 2007) reported a low intake of dairy products and scored consumption negatively, however interpretation varies between scores with some giving preference to fermented dairy or low-fat dairy or goats/sheep products (Guo et al. 2017). The current score excludes dairy as an item, a decision that is supported by the recent meta-analysis of 29 prospective cohort studies demonstrating neutral associations between dairy products and CVD, including separate analyses for low-fat, fermented, cheese, and yogurt (Trichopoulou et al. 2003). One of the limitations of this score is the lack of adjustment for energy intake and the allocation of equal weighting for all index components. Ideally the items that have greater impact on health should be ascribed greater weight. However, this would require published data (relative risks) on the relative contribution of each dietary component to the same health outcome, which does not yet exist. Secondly, it

would ignore the existing correlations and interactions between the individual dietary components, negating the original reason to investigate dietary patterns. Thus, relative attribution is not realistically viable.

#### **Implications of findings: How Mediterranean Diet Score relates to BFF trial results**

On a practical level, this study demonstrates that changes in diet quality at an individual level can be assessed easily in everyday clinical practice using the Mediterranean Diet Score. The consensus from observational studies in the general population is that an increment of one point of the Mediterranean Diet Score equates to 5% reduction in risk of CVD, with evidence from several meta-analyses: one point increment in Trichopoulou Mediterranean Diet Score (Rosato et al. 2017) using mean intake cut points showing 5% reduction in MI (RR 0.95, 95%CI 0.92 to 0.99, 6 studies, (Sofi et al. 2014)); 2-point increase in adherence score reducing risk of CVD by 10% (RR 0.9, 95%CI 0.87 to 0.92,  $I^2=38\%$ , 20 studies, (Martínez-González et al. 2017) and RR 0.89, 95%CI 0.86 to 0.91,  $I^2=76\%$ , 27 studies, (Estruch et al. 2018)). In the current trial, participants demonstrated a 3-point improvement in Mediterranean Diet Score. Extrapolating the evidence from the general population, this could potentially translate into a 15% reduced risk of CVD. Meanwhile, comparing the current study directly with findings from the PREDIMED trial, the current study conservatively yielded a 2-point increase (lower confidence interval), whilst PREDIMED's 2-point increase produced an 11% relative risk reduction in CVD, although caution must be exercised as this was the lower confidence interval of a composite endpoint (Jacobs et al. 2018). If we assume that the Mediterranean Diet

Score is a continuum, and that the points are equal in their disease effects, then any improvement is valid, and individuals can aim for small improvements within the routine care setting.

Alternatively, it has been postulated that the impact on CV risk reduction may only exist above a certain threshold, such as Mediterranean Diet Score over 5, as this has implications for populations in non-Mediterranean regions where scores are estimated to be 2 (Jacobs et al. 2018). Using those criteria, in the current study 86% of participants in Mediterranean Portfolio group had MDS>5 at month 6, compared to 61% in low saturated fat group, meaning that impact would be achieved. The absolute Mediterranean Diet Score would therefore also be of importance; again, the results in the current study with mean Mediterranean Diet Score 9.5 are comparable with PREDIMED of 10, even though it was conducted in a non-Mediterranean country.

As a dietary adherence tool, this score proved fit for purpose documenting a significant improvement in adherence to the Mediterranean Diet in the intervention group and a stable score in the control group. The changes were consistent with increased fibre intake, as calculated from the food diaries. The score highlighted the individual components that contributed most to the changes - olive oil, nuts, cake/biscuits, and highlighted areas of significant improvement where participants met targets for consumption of nuts, legumes, fish and cake. The increase in total score was substantial (2 or more Mediterranean Diet Score points) in 76% of

participants in the intervention group (a similar majority was observed in PREDIMED of 55% (Jacobs et al. 2018)), with a wide variation (see in (de Lorgeril et al. 1999)) implying that the changes came from a range of food combinations for different participants and is therefore reflective of the dietary pattern.

Researchers have debated over which specific foods are responsible for the beneficial outcomes observed. In the Lyon Heart Trial, the alpha-linolenic acid from the rapeseed oil spread was inferred as the key factor rather than the Mediterranean diet (although it also includes alpha-linolenic acid sources: soya beans, flaxseed and walnuts) (Appel & Van Horn 2013). Similarly, commentaries on the PREDIMED trial pointed towards the items provided to participants (olive oil and nuts) as the key components in the way the participants implemented their assigned treatments (Jacobs et al. 2018). This was disputed by the research group as legume and fish intakes increased in both Mediterranean diet intervention groups, and further examination revealed that changes to intakes of nuts and extra virgin olive oil only accounted for 25% of overall change in the total Mediterranean Diet Score (Grosso et al. 2015). Both arguments are supported by a meta-analysis of 11 studies using pooled risk analysis for single food components which reported that the protective effect of the Mediterranean diet appeared to be most attributable to olive oil (RR 0.83, 95%CI 0.77 to 0.89,  $I^2=0\%$ ), fruit (RR 0.88, 95%CI 0.81 to 0.96), vegetables (RR 0.87, 95%CI 0.77 to 0.98) and legumes (RR 0.90, 95%CI 0.83 to 0.98) (Trichopoulou et al. 2009). Whilst examination of a different cohort (the Greek section of EPIC) revealed that high consumption of plant foods accounted for 37% of the

reduction in mortality (vegetables 16%, fruit and nuts 11%, legumes 10%) compared to 17% from low meat (Marzel et al. 2018). These findings are consistent with empirically derived dietary pattern data (using hierarchical clustering method) in predominantly white men aged 56 years from Swiss HIV Cohort Study where a positive correlation was found between elevated total cholesterol ( $>5.2\text{mmol/l}$ ) and the frequency of combined consumption of meat, refined/milled grains, carbonated beverages, and coffee (Funderburg & Mehta 2016). Thus, it would appear to be difficult to isolate any individual food as beneficial or detrimental but rather the complete dietary pattern is responsible, with a degree of variability that lends itself to individual interpretation and personal preference.

## Recommendations for future research

The current BFF study has confirmed the causal nature of the relationship between diet and LDL-cholesterol reduction within the HIV setting. Meanwhile, the SATURN-HIV study placed oxidised LDL-cholesterol on the causal pathway of CVD progression in HIV infection and demonstrated that statin therapy reduced vascular inflammation (FERENCE et al. 2017). The combined evidence supports the consensus in the general population that dietary intervention reduces LDL-cholesterol to reduce the risk of CVD (Gilbert et al. 2015). Whilst the ongoing REPRIEVE trial of statin treatment on clinical endpoints should further enhance understanding on mechanisms of atherosclerosis in HIV (Ridker et al. 2008; Narla et al. 2009). However, the focus cannot remain solely on LDL-cholesterol reduction as the

solution to rising CVD. In the general population, the JUPITER trial showed that 50% residual cardiovascular risk remained despite treatment to target levels of LDL-cholesterol with statin therapy, highlighting the need to target alternative pathways to reduce CV risk (Shivappa et al. 2014). Therefore, future studies need to explore the possibility of preventing atherosclerosis through other mechanisms such as modulation of inflammation and intestinal immunity.

In the current study inflammatory markers were elevated but remained unchanged by the dietary intervention. Future work could evaluate the inflammatory potential of the dietary intervention by applying the Dietary Inflammation Index retrospectively in post-hoc analysis and exploring potential overlap between the Mediterranean Diet and anti-inflammatory diet. The Dietary Inflammatory Index was developed to appraise the inflammatory potential of the diet and addresses two of the limitations of the Mediterranean Diet Score. Firstly, it is literature-derived and population based, because it involved collation of the effect of 45 dietary parameters on six inflammatory biomarkers from 1,943 published studies (Shivappa et al. 2014). It is therefore not dependent on a single study or within similar populations. Food parameters (such as garlic, ginger, and flavonoids) were scored negatively for their anti-inflammatory or positively for their pro-inflammatory effect, producing a continuum of increasing inflammatory effect from -9 to +8 (García-Arellano et al. 2018). Meta-analyses have demonstrated an association between the Dietary Inflammatory Index and increased risk in all-cause mortality (RR 1.23, 95% CI 1.16 to 1.32,  $I^2=71\%$ , 12 studies) (Shivappa, Godos, et al. 2018), and CVD (8% increased



risk for each one-point increase in Dietary Inflammatory Index score, RR=1.08, 95%CI 1.04 to 1.12,  $I^2=75\%$ , 14 studies) (García-Arellano et al. 2015). Although many of the studies were cross sectional, the meta-analysis included the PREDIMED study showing direct prospective evidence that a pro-inflammatory diet is associated with a higher risk of CVD (Shivappa, Bonaccio, et al. 2018). A difference in Dietary Inflammatory Index between Mediterranean diet and low-fat dietary intervention groups has not been seen in other trials (Wang et al. 2017), despite a correlation between reduction in Dietary Inflammatory Index score and reduction in IL-6 in the whole cohort ( $r=0.34$ , 95%CI 0.05 to 0.56). Characterisation of an anti-inflammatory diet with low Dietary Inflammation Index scores has been described as being mainly plant based, rich in vegetables, fruit, nuts, legumes and fish, and low in meats, dairy and bakery; thus, has similarities to the Mediterranean diet.

Advances in metabolomics may help to develop future understanding of the inflammatory mechanisms underlying CVD, for example with further exploration into the role of ceramides. In PREDIMED, plasma ceramide concentrations were associated with incident CVD, which was modified by the Mediterranean Diet intervention (Yamashita et al. 2016; El-Far & Tremblay 2018). Ceramides are carried by LDL-cholesterol and become embedded in the arterial wall of atherosclerotic plaques where they attract inflammatory immune cells to the plaque site, disrupting endothelial function, preventing normal vasodilation and vasoconstriction.

With increasing evidence of the role of gut microbiota in health and disease, future studies are required to explore the interactions between diet, HIV infection, gut microbiome, inflammation, and atherosclerosis. Accumulating evidence suggests that lower diversity in gut microbiota is associated with poorer health, particularly inflammatory diseases (Yamashita et al. 2016). Specific gut metabolites, such as trimethylamine oxide (TMAO), elevated by red meat consumption, are involved in atherosclerotic lesion formation (D'Angelo et al. 2017). It is understood that HIV is associated with an altered gut microbiome that is not consistently restored with effective ARV treatment (De Filippis et al. 2016). Meanwhile, significant associations have been found in the general population between high adherence to a Mediterranean diet (rich in legumes, fruit and vegetables) and a beneficial microbiome-related metabolomic profile, indicated by lower trimethylamine oxide (TMAO) levels (De Filippis et al. 2016; Garcia-Mantrana et al. 2018), increased levels of faecal short-chain fatty acids (acetic, propanoic, and butanoic acids), increased proportion of fibre-degrading bacteria (Bacteroidetes) (Mitsou et al. 2017), higher Bifidobacteria and lower *Escherichia coli* counts (El-Far & Tremblay 2018). Clinical trials using probiotics in virologically suppressed HIV patients have shown promising reductions in CD4 T-cell activation, microbial translocation, systemic inflammation, and specific inflammatory risk markers for CVD (Trichopoulou et al. 2014). Future studies should explore mechanisms that may be independent of lipid lowering effects, such as modulation of gut microbiota to suppress atherosclerotic lesion formation with interventions such as dietary prebiotics, probiotics or faecal microbiota transplantation (FMT).

To date, the applicability of the Mediterranean diet within non-Mediterranean countries has not been established. The Greek pioneers of the Mediterranean diet acknowledge that exploration of what it means in the context of other countries with distinct cultural diets and lifestyles is still required (Jacobs et al. 2018). This study provides some insight into the interpretation and implementation of the diet by a variety of individuals from different ethnicities living in northern Europe. Moving forward, an international group of researchers have examined the feasibility of replicating the PREDIMED study in the United States to see if the diet is transferable to the American setting (Patel et al. 2018) but acknowledge that pilot work is required to identify lower cost, high yield intervention methods before a full study can be undertaken. With regard to the HIV population, the cardioprotective effects of the Mediterranean Diet now need testing in areas of high HIV prevalence, such as the African setting, due to the economic burden of rising incidence of non-communicable diseases such as CVD.

In summary, this study provides evidence of efficacy that the Mediterranean Portfolio diet reduces cardiovascular risk factors in people with HIV dyslipidaemia. The piloting process prepares the ground for pragmatic dietary intervention trials to demonstrate effectiveness and sustained dietary change in the long-term. This would include recruiting a wider population (both geographically, and those with comorbidities and associated medication), and as the literature suggests, may benefit from inclusion of physical activity and enhanced support strategies. Further examination of the underlying mechanisms of the role of diet in HIV related atherosclerosis are also

warranted, including the role of inflammation and gut microbiome. This echoes some of the research agenda questions compiled following a 4-year working party of over 100 stakeholders to address key unanswered topics around non-communicable diseases in Africa. They included: 'What is the impact of a low cholesterol diet on CVD outcomes among people living with HIV?' and 'What is the impact of lifestyle counselling on CVD and its risk factors in people living with HIV in LMICs?' (Patel et al. 2018).

## APPENDICES

### Appendix 1. Changes in lipids in ART switch studies

Table. Changes in plasma lipids reported in trials of switching antiretrovirals.

Where a significant difference was reported, the mean is shown in mmol/l.

Source: BHIVA Guidelines, August 2016, Table 8.3, page 124 (Churchill et al. 2016)

Class	Drug	TC	LDL	HDL	TC/HDL	TGs	Ref
<b>NRTIs</b>	Kivexa to Truvada <sup>1</sup>	-0.47	-0.23	NS	NS	NS	[81]
<b>NNRTIs</b>	EFV to RAL	-0.36	NS	NS	—	NS	[82]
	EFV to ELV/c	-0.21	-0.20	NS	NS	NS	[83]
	EFV to NVP	NS	-0.34	NS	—	NS	[84]
	EFV to RPV	-0.44	-0.21	NS	NS	-0.29	[85]
	PI/r+2NRTI to RPV/TDF/FTC	-0.62	-0.41	-0.07	-0.35	-0.63	[86]
	EFV to ETV	-0.64	-0.58	NS	NR	NS	[87]
<b>PIs</b>	ATV/r to ATV <sup>2</sup>	-0.34	NS	NS	-0.24	-0.36	[88]
	ATV/r to RAL	NS	—	—	—	NS	[89]
	LPV/r to ATV/r	-0.39	NS	NS	—	-0.55	[90]
	LPV/r to RAL	-0.44	—	—	—	-0.49	[89]
	LPV/r to RAL <sup>3</sup>	-0.8	NS	—	—	-0.90	[91]
	ATV/r to ELV/c	NS	NS	NS	NS	-0.42	[92]
	DRV/r to ELV/c	NS	NS	NS	NS	NS	[92]

NS: difference not statistically significant; ATV/r: atazanavir/ritonavir; DRV/r: darunavir/ritonavir; EFV: efavirenz; ELV/c: elvitegravir/cobicistat; ETV: etravirine; LPV/r: lopinavir/ritonavir; NVP: nevirapine; RAL: raltegravir; RPV: rilpivirine; RPV/TDF/FTC: rilpivirine/tenofovir-DF/emtricitabine

<sup>1</sup> With PI/r; <sup>2</sup> Atazanavir plus abacavir/lamivudine backbone; <sup>3</sup> Powered for a change in lipids

## Appendix 2. PRECIS domains and rationale

Domain	Score	Rationale
Eligibility - Who is selected to participate in the trial?	3	Broad inclusion criteria: adults, stable on ARV treatment >6 months, LDL>3mmol/l (routine test). Participants are very similar to those who would receive dietary intervention in clinic. People excluded are those on drugs or with comorbidities that might influence lipid levels (lipid lowering agents, diabetes, liver, renal, hypothyroidism, familial hyperlipidaemia, pregnancy) and contraindications to research in general (psychiatric disorder, participation in another study/diet, inability to understand printed materials).
Recruitment - How are participants recruited into the trial?	4	Patients recruited from 3 different HIV clinics, approached during routine care, with enrolment and randomisation conducted at routine clinic visits. Proactive aspect to speed up recruitment - electronic blood results reviewed to identify patients who were eligible (LDL>3mmol/l), who would then be approached at next appointment - achievable in usual care but requires more resources.
Setting - Where is the trial being done?	5	Participant is seen in usual clinic environment or clinic room booked in research building, depending on availability. Three recruitment sites used, typical of HIV care within NHS. Participants represent socioeconomic and ethnic mix of population.
Organisation - What expertise and resources are needed to deliver the intervention?	2	Identical to usual care: provider of intervention is specialist HIV dietitian, and organisation is modeled on routine care. However trial used increased resources for: provision of unusual food items, higher number of follow up visits, and length of consultations. These differences were explicit features of the intervention.
Flexibility - How should the intervention be delivered?	2	Dietary intervention was described in detail in the protocol (e.g. 2g plant stanols/day), and foods were introduced at specific timepoints (fibre and soya at month 1). Restrictions placed on co-interventions that would enhance any intervention effect (lipid lowering drug/food).

		How the intervention was delivered was flexible and at the discretion of the provider, e.g. in the style of motivational interviewing, with adaptations for culture, personal preferences, affordability of foods, and family dynamics.
Flexibility - What measures are in place to make sure participants adhere to the intervention?	4	Participants are given dietary advice and encouraged to follow it, but it is their decision as to what they change or don't. Intervention involved motivational interviewing techniques to encourage adherence to intervention. Adherence to diet is monitored for outcome purposes, but participants not excluded if adherence is poor.
Follow-up - How closely are participants followed-up?	2	Usual care was every 3 months when trial was designed. Trial involved additional follow-up at 1 month. Data collection was more extensive. Visits were longer.
Primary outcome - How relevant is it to participants?	3	LDL-cholesterol is measured in routine care, is understood by patients, is a surrogate for CVD, and used in meta-analyses. Ideal outcome measure would be CV events, but event rate is too low in this young population. Outcome of importance to patients would be waist circumference.
Primary analysis - To what extent are all data included?	5	ITT analysis with all available data, including sought out clinical data where research data was missing due to drop out.

## Appendix 3. Participant consent form

Patient Identification Number for this trial:

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### CONSENT FORM

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Title of Project: **Best Foods For your heart**

Name of Researcher: **Clare Stradling, Chief Investigator**

Please initial boxes

1. I confirm that I have read and understand the information sheet dated 2 Dec 2103 (version **2**) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from **University of Birmingham**, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that the consultations with the dietitian may be recorded for the purpose of monitoring quality and that all the information will be kept strictly confidential and that the recording will be destroyed at the end of the study. ☐
5. I agree to my GP being informed of my participation in the study. ☐
6. I agree to take part in the above study. ☐
7. I give consent to be contacted about taking part in an interview about my involvement in the study. I understand that by ticking this box I am consenting to be contacted but I am under no obligation to take part in the interview. You can still take part in the study if you choose not to tick this box. ☐

---

Name of Participant

---

Date

---

Signature

---

Name of Person  
taking consent.

---

Date

---

Signature

Please tick if you wish to receive a report of the study findings when it is complete. ☐



## Appendix 4. Patient information sheet

### Participant Information Sheet

We are inviting you to take part in the **B**est **F**oods **F**or your heart ❤️ Trial. Before you decide to take part, we would like you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear. The research dietitian will go through this with you at your next clinic appointment and answer any questions you may have.

#### What is the purpose of the study?

This study will look at two different diets and their effect on blood cholesterol levels in people with HIV infection. Previous research has shown that these two different diets can lower blood cholesterol levels and reduce the risk of death from heart disease in people without HIV infection. We want to know if the same is true for people with HIV infection, who are at greater risk of heart disease. Therefore this study could potentially aid the development of new treatment options for people with raised cholesterol levels without the need for additional drugs. Another important aspect of this study is to look at how to implement these diets in every day life.

#### Why have I been invited?

You have been selected because you have raised cholesterol levels in your blood.

#### Do I have to take part?

No, it is up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form with a research nurse. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

#### What will happen to me if I take part?

This trial has been designed to follow your usual pattern of care for 1 year. Most of your research visits will happen at the same time as your routine blood or doctor appointments. Three of these visits will last approximately 1 hour, as they involve consultations with the dietitian, the other two will last approximately 30 minutes. You will only need to attend one additional visit (at month 1) lasting between 20 and 40 minutes. For this visit you will receive a £15 gift voucher to compensate you for your travel. You will also be contacted by phone on two occasions to discuss your progress.

At the same time as your routine clinic bloods, we will take additional samples of blood and urine to measure your sensitivity to insulin, blood fats, and biomarkers of what you are eating. You will need to attend fasted for the tests to be accurate; no food or drinks for the 12 hours before the blood test, only water. One blood sample will be drawn into 4 tubes to be sent to the laboratory for analysis. You will collect the urine at home in a container that we will provide, and bring it in to clinic.

On three occasions during the trial we will measure your weight, height, waist circumference, body fat, and health of your arteries. To test the health of your arteries you need to lie down flat on a couch for 10 minutes, whilst a sensor is placed on your neck and a cuff round each arm

and leg in turn to measure the blood pressure of both your arms and legs. The other tests will involve standing on scales in bare feet.

You will be given advice on how to follow either diet 1 or diet 2. Both diets involve changing the type of fat that you use. A computer will choose which diet you are given, by a process called randomisation, which is rather like tossing a coin. This type of study is known as a randomised controlled trial. Sometimes we don't know which way of treating patients is best. To find out, we need to compare different treatments. In this trial we will put people into groups and give each group a different diet. The results are compared to see if one is better. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly). You will have a 50:50 chance of getting diet 1 or diet 2.

### **What will I have to do?**

As with any study, you will need to attend all the scheduled visits (at month 0, 1, 3, 6, 12) and be available for telephone consultations (at month 2 and 4). Specific to this study, you will need to change some of the foods you buy and eat in order to follow the diet. You will be given practical advice on shopping and cooking to help you make these changes. For the first 6 months you may be supplied with some of the recommended foods that are considered to be expensive or difficult to find.

At the beginning and end of the trial you will be asked to complete questionnaires about how you manage with day-to-day activities, study evaluation questionnaires, a diary of everything you eat and drink for 3 days, and wear an Actigraph (a watch size device, like a pedometer) on your belt for 7 consecutive days to measure your body movements. The food diary and Actigraph are returned at your next visit. The questionnaires should take no longer than an extra 20 minutes in the clinic. At every visit you will be asked to complete food scores or questionnaires about the types of food you have eaten in the previous month. This should take no longer than an extra 5-10 minutes in the clinic. Some of your consultations with the dietitian may be audio recorded for quality purposes; they will be destroyed at the end of the study. During the study period, you will also be asked to take a couple of photographs that will help you share how you felt about changing your diet and tell your story of what it was like for you during these 6 months.

### **What are the possible disadvantages and risks of taking part?**

We are not aware of any risks connected with this study. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against University of Birmingham, who are sponsoring the study, but you may have to pay your legal costs.

### **What are the possible benefits of taking part?**

We cannot promise the study will help you but the information we get from this study will help improve the treatment of people with HIV infection. We do not want you to incur any personal costs as a result of taking part in the study. A gift voucher will be given for attending the additional session to offset your travel costs. You may receive supplies of certain foods that are required for the study, in the first 6 months.

### **What happens at the end of the study?**

At the end of the study you will return to usual care from your HIV clinic. Food samples will not be continued after the end of the study.

**What if there is a problem?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions 0121 424 0644. If you remain unhappy and wish to complain formally, you can do this through the normal NHS complaint mechanisms, Patient Advice and Liaison Service (PALS) on 0121 424 1212 or email at [pals@heartofengland.nhs.uk](mailto:pals@heartofengland.nhs.uk)

**Will my taking part in this study be kept confidential?**

Yes. We will follow ethical and legal practice in accordance with the Data Protection Act 1998. All information that is collected about you during the course of the research will be kept strictly confidential. All information collected during the study will be identified by a unique code, rather than your name, so that you cannot be recognised. Personal details will not be included in analysis, or in publications or reports.

**What if relevant new information becomes available?**

Sometimes we get new information about the treatment being studied. If this happens, your research dietitian will tell you and discuss whether you should continue in the study. If you decide to continue in the study she may ask you to sign an agreement outlining the decision. If the study is stopped for any other reason, we will tell you and arrange your continuing care.

**What will happen if I don't want to carry on with the study?**

You can withdraw from treatment but keep in contact with us to let us know your progress. Information collected may still be used.

**What will happen to the results of the research study?**

At the end of the study we will send a report describing the final results to everyone involved in the study. We will also write reports for professional medical journals, present the results at conferences, and publicise them through voluntary groups. You will not be identified in any report or publication.

**Who is Organising and Funding the Research?**

The study is organised by a research team at the University of Birmingham and funded by The National Institute for Health Research.

**Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by West Midlands Solihull Research Ethics Committee.

Further information and contact details: Clare Stradling  
MIDRU, Birmingham Heartlands Hospital  
Bordesley Green East, Birmingham B9 5SS  
Telephone: 0121 424 2881 [Clare.stradling@heartofengland.nhs.uk](mailto:Clare.stradling@heartofengland.nhs.uk)

***Thank you for your interest in the **Best Foods For your heart** ♥ study.***

## Appendix 5. Mediterranean Diet Score

	✓ <input type="checkbox"/> X or number
1. Do you use olive oil as your main fat for cooking?	
2. How much olive oil do you eat each day? (for frying, salads, and out of house meals)	
3. How many servings of vegetables do you eat each day? (1 serving = 80g)	
4. How many servings of fruit do you eat each day? (natural fruit juice can only count as one serving)	
5. How many servings of red meat, hamburgers, meat products, ham, or sausage each day? (1 serving = 100-150g)	
6. How many servings of butter, margarine, or cream do you eat each day? (1 serving = 12g)	
7. How many carbonated or sugar-sweetened beverages do you drink each day? (e.g. squash, fruit drink with added sugar, lemonade, cola)	
8. Do you drink 3 or more glasses of wine each week?	
9. How many servings of beans or lentils do you eat each week? (1 serving = 150g)	
10. How many servings of fish or shellfish do you eat each week? (1 serving = 100-150g fish or 5 prawns, mussels, etc)	
11. How many times do you eat commercial (not homemade) pastries, cakes, cookies, biscuits, or sweets each week?	
12. How many times do you eat nuts each week? (1 serving = 30g)	
13. Do you prefer to eat chicken, turkey or rabbit instead of pork, beef, lamb, mutton, hamburger, or sausage?	
14. How many times per week do you eat pasta, rice or maize with a tomato sauce (made with olive oil, onion, tomatoes) at least twice a week?	
Total	

## Appendix 6. Process Evaluation Questionnaire 1.

Please tick one box for each statement to show whether you agree or disagree.

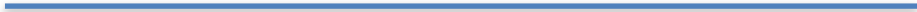
	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
I am certain that I can eat a healthy diet, even if things get difficult					
I am motivated to lower my cholesterol levels	Not motivated				Extremely motivated
I am confident in my ability to lower my cholesterol levels	Not confident				Extremely confident
Eating low fat foods will....					
Help me be a role model for my family					
Make me feel good					
Keep my body in good shape					
Improve my health					
Allow me to eat more foods without getting too many calories					
People (family/friends/co-workers) who are close to me....					
Encourage me to eat healthy low-fat foods					
Remind me to eat healthy foods					
Criticise me for eating healthy, low-fat foods					
Eat unhealthy high-fat foods in front of me					
Offer me unhealthy high-fat foods					

On a scale of 1 to 10, how ready are you to make a change?


Please mark the line for each question to indicate your current position.

Where you feel you are at the moment.

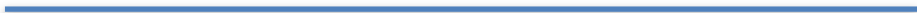
1) How important is it for you to change your diet / make changes to what you eat?

1      2      3      4      5      6      7      8      9      10  
Not important  very important

2) How confident are you that you would succeed at changing what you eat?

1      2      3      4      5      6      7      8      9      10  
Not confident  confident will succeed

3) How ready are you to start making a change in what you eat?

1      2      3      4      5      6      7      8      9      10  
Not ready to change  already changing

4) In this research study, which group would you prefer to be allocated to?	New complex diet	Old easy diet	Don't mind
---	------------------	---------------	------------

5) What do you hope to get out of being a part of this study?

.....  
.....

6) In what way do you expect to change over the next year?

.....  
.....

6) I'm doing this study because .....

***Thank you for your comments.***

## Appendix 7. Process Evaluation Questionnaire 2.

	Strongly disagree	Disagree	Agree	Strongly agree	Not applicable, don't know, didn't use
The leaflets were easy to understand					
The leaflets provided me with useful information					
The recipes were time consuming					
I enjoyed accessing the websites					
Keeping a food diary was difficult					
I now keep a record of what I eat					
I now set myself nutrition goals					
The quality of the telephone support was good					
The website links provided me with useful information					
The quality of support from the dietitian was good					
I am satisfied with the diet group I was allocated to					
I would prefer being in a programme that meets in a group than one that meets one-to-one					
I would prefer more clinic visits / support					
The information and advice helped dispel myths about nutrition and heart disease					
My involvement in the diet trial was enjoyable					
Shopping and cooking more time consuming					
These new foods were easy to find in shops					

## Diet Intrusiveness Scale

Please show how this new way of eating interferes with your life on a scale of 1 to 5, by circling a number in the frequency column and the impact column.

1 2 3 4 5

never always

1 2 3 4 5

low impact high

Frequency – How often this is a problem for you	My new diet....	Impact – How it interferes with your life
1    2    3    4    5	...interferes with my social life	1    2    3    4    5
1    2    3    4    5	...is difficult to cook & prepare	1    2    3    4    5
1    2    3    4    5	...makes it difficult to be spontaneous	1    2    3    4    5
1    2    3    4    5	...is too complex to stick to	1    2    3    4    5
1    2    3    4    5	...restricts my ability to travel	1    2    3    4    5
1    2    3    4    5	...interferes with my work	1    2    3    4    5
1    2    3    4    5	...makes it harder to meet friends	1    2    3    4    5
1    2    3    4    5	...interferes with my relationships	1    2    3    4    5
1    2    3    4    5	...doesn't taste as good	1    2    3    4    5
1    2    3    4    5	...is difficult to buy suitable foods	1    2    3    4    5



Please circle one or more options:

When it comes to cooking, I would describe myself as:

Novice with little or no cooking experience

Comfortable with basic cooking

Comfortable with following a recipe

Happy to make things up

Professional chef

My involvement in the trial has encouraged me to:

Buy more healthy foods

Grill, bake, steam or microwave instead of frying

Cook meals from scratch, rather than use ready meals

Discuss healthy eating with others

Eat as I did before

What has been your overall experience of the trial?

Describe the strengths of the programme.....

.....

.....

Describe the weakness of the programme.....

.....

.....

Which advice / support / materials did you find particularly useful?

.....

.....

What are your suggestions for improvement?

.....

.....

Thank you for your comments

Just one more question please! ☺

In the last week, please circle the days that you have:

Taken the plant stanol drink?

Su M Tu W Th F Sa

Eaten a handful of nuts?

Su M Tu W Th F Sa

Eaten at least one portion of soya food?

Su M Tu W Th F Sa

Eaten a portion of oily fish or beans or pulses?

Su M Tu W Th F Sa

Eaten at least one portion of oats or barley?

Su M Tu W Th F Sa

**Appendix 8. Table showing number of patients screened, eligible and enrolled, with primary reasons for exclusion.**

Site	Screened	Eligible	Reasons excluded due to ineligibility	Enrolled	Reasons excluded from enrolment	Randomised	Reasons excluded from randomisation
C&W	26	24	1 on diet 1 language barrier	17	3 no contact 2 declined 1 moving 1 statin	13	1 DNA 1 LDL <3mmol/l 1 on diet 1 moving
HEFT	882	145	358 LDL<3mmol/l 147 on statin 92 not on ARVs or recent change 54 VL >40 copies/ml 37 comorbidities 18 on different diet 12 pregnant or MOD or in other study 11 language barrier 8 psychiatric disorders, drug/alcohol dependency	50	68 declined: 21 too busy, 8 too far, 7 won't change diet, 6 can't commit 5 fasting difficult, 5 dislike research, 5 want specific diet, 4 stressed, 4 not interested, 3 financial 27 no contact or DNA	40	5 DNA or no food diary 3 LDL <3 mmol/l 2 on diet
UHB	25	13	6 LDL<3mmol/l 1 statin 2 Allergic to nuts 2 Dislikes nuts 1 gastric band restricting intake	10	3 declined: 2 not interested in research 1 believes current diet is good	7	1 DNA 1 on diet 1 on statin

**Key:**

C&W City of Coventry Clinic

HEFT Birmingham Heartlands Hospital

LDL low density lipoprotein cholesterol

VL HIV viral load

UHB Queen Elizabeth Hospital

DNA did not attend

ARVs antiretroviral treatment

MOD military patient

## Appendix 9. Socio-economic status categories.

National Statistics Socio-Economic classification (NS-SEC) integrates social class based on occupation and socio-economic groups. The NS-SEC schema is constructed on occupation, employment status (e.g. self-employed), and establishment size, to produce an 8-class system:

1 higher managerial and professional occupations	1
2 lower	1
3 intermediate occupations	2
4 small employers and own account workers	2
5 lower supervisory and technical occupations	3
6 semi routine occupations	3
7 routine occupations	3
8 never worked and long-term unemployed	3

A further category (9) can be used for full time students but was omitted in this study as all the participants who were studying also had jobs.

Ref: National Statistics SEC User Manual 2005 Palgrave Macmillan HMSO

Using Derivation tables based on SOC2000

In the literature, we are more familiar with a 5-class system, where traditionally SE groups were based on social class:

- |     |   |                              |
|-----|---|------------------------------|
| I   | A | professional                 |
| II  | B | managerial and technical     |
| III | C | skilled – manual, non-manual |
| IV  | D | partly skilled               |
| V   | E | unskilled                    |

And the Census 2011 used Social Grade model:

- |    |   |
|----|---|
| AB | high and intermediate managerial, professional    |
| C1 | supervisory, junior clerical, admin, professional |
| C2 | skilled manual                                    |
| DE | unskilled manual, UE                              |

However, it was unclear how these related to the 8-class system.

As the 8-class system was unwieldy and yielded multiple cells with small values, the 8-class schema was collapsed into a 3-class version, as validated by Rose and O'Reilly 1998:22 The ESRC Review of Government Social Classifications, London, ONS, HMSO report, where 1=1&2, 2=3&4, 3=5-8.

## Appendix 10. Patient resources used for intervention

As the intervention is complex, it is standardised by function (goals negotiated tailored to socio-economic setting, family dynamics, food and cultural preferences), not form (precise components or resources). Therefore, providing a detailed step by step prescriptive account of each visit is neither feasible nor appropriate (Hawe et al. 2004). The principle of dietary intervention is to educate, empower and motivate participants to make changes towards a Mediterranean eating pattern and is outlined here.

Planned Action	Practical advice: aim & content	Resources given
Baseline visit: enrolment.		
To coincide with patient's blood appointment. All measurements and interaction are exactly the same for all trial recruits at this visit.		
Allocation visit: randomisation		
The Allocation visit is conducted 1-2 weeks after the Baseline visit, to coincide with their routine Dr clinic visit. The intervention starts here.	<p>Group 1</p> <p>To achieve saturated fat intake &lt;10% of energy intake.</p> <p>Content: Discussion on sources of saturated fat &amp; photos of food swaps using Ultimate Cholesterol Lowering Plan (UCLP) flipchart produced by HEART UK, p6 with modifications, deleting soya cream in latte and crumble.</p> <p>Tips for cutting down on fatty and processed meats, butter, lard, ghee, fried foods, full cream milk, cakes, chocolate and pastries. Fat</p>	<p>'Reducing saturated fat' A4 sheet produced in-house, using material from out of print resources: Saturated fat made simple by Food Standards Agency, and Healthy eating for a healthy heart by British Heart Foundation 2009.</p> <p>'Cheese facts' A4 sheet from British Heart Foundation: G186e_BHF_Eating well_Cheese facts-download.pdf</p>

	content of different cheeses discussed using leaflet.	
<p>Motivational interviewing style to include:</p> <p>Engaging – introductions, examining expectations, concerns &amp; aim of session.</p> <p>Focus – decisional balance, identify motivators and barriers, reasons for making change, readiness to, and importance of making changes, explore experience of keeping food diary.</p> <p>Evoke – self-efficacy, strengths, abilities, talents, confidence on succeeding, building on past experiences; help participant to verbalise arguments for change – desire, ability, reasons, need to change, commitment, activation.</p> <p>Planning – summarise change talk and commitment language, agree specific changes using affirmation.</p>	<p>Group 2</p> <p>To achieve saturated fat intake &lt;10% of energy intake; &gt;5 portions of fruit and vegetables daily; 2 portions oily fish weekly; 2g/d plant stanols; 57g/d tree nuts (2 handfuls).</p> <p>Content: motivational interviewing style consultation using UCLP flipchart for pictorial prompts to discuss atherosclerosis, cholesterol types, Eatwell plate, reducing saturated fat, sources of fat p6, stanols and nuts p10. Use of completed food diary to guide dietary changes relevant to individual's current intake and situation.</p>	<p>Nuts in a serving (Australian Tree Nut Industry 2013) – photos of different nuts.</p> <p>BDA fact sheets: Stanols and sterols (Jan 2012); Omega 3 (fish) (April 2013).</p> <p>Five a day Just Eat More from NHS.uk/5aday (Aug 2008) – photos of portions sizes of fruit and vegetables.</p> <p>Supply of unsalted mixed nuts, tinned oily fish and 50ml cholesterol lowering drinks.</p>

Month 1		
Intervention visit 2, conducted 1 month after allocation, at time convenient for participant.	<p>Group 1</p> <p>Reinforce saturated fat intake &lt;10% of energy intake.</p> <p>Content: explain food labelling, traffic light system, prioritising low saturated fat.</p>	<p>'Food labelling' by Food Standards Agency until it was discontinued, then changed to 'This label could change your life' (BHF 2014).</p> <p>NHS Change 4 Life website link: 'cut back on fat', 'cut down on fat when you shop', 'food labels'.</p>
<p>Motivational interviewing to include:</p> <p>Engaging - Celebrate changes made, how they were achieved; examine difficulties &amp; attempts to overcome;</p> <p>Focus – reinforce changes and reasons for change;</p> <p>Evoke - change talk, offer alternatives for foods/options that haven't worked according to food preferences;</p> <p>Planning – goal setting.</p>	<p>Group 2</p> <p>Reinforce previous aims (saturated fat intake &lt;10% of energy intake, 57g nuts &amp; 2g plant stanols daily). Further advice to achieve 15-20g/d soluble fibre; 15g/d soy protein;</p> <p>Mediterranean style diet with emphasis on fruit, vegetables, olive oil, fish, beans, grains, with meals cooked from unprocessed foods.</p> <p>Content: review dietary goals and motivational triggers using UCLP flipchart p9, 11, 12</p>	<p>UCLP tear off sheets (2012 Alpro) showing portions of fruit, vegetables, fish, soya, stanols, nuts, oats, beans to aim for.</p> <p>Mediterranean diet (2009 Oldways).</p> <p>Mediterranean diet – individual weekly portion count, from patient.co.uk.</p> <p>Supplies of: oats, pearl barley, lentils, beans, flaxseed, olive oil, soya milk/yogurt/dessert, tofu, meat substitutes.</p>
Month 2		
Telephone follow up	Group 1: evaluate quantity, type, timing & cooking methods of fruit and vegetable consumption in typical day.	
	Group 2: evaluate quantity, type, timing & cooking methods of fruit and vegetable consumption in typical day.	



	Review progress and encourage, Support and encouragement, reinforce previous advice, exploring difficulties and barriers to change.	
Month 3		
Reinforce dietary intervention	Group 1 Reinforce previous aim. Content: using photos of different brands of margarine establish which one is bought, link to previous session on food labelling aiming for saturated fat <15g/100g. Low fat recipes.	Low fat recipe booklet produced in house. Recipes on nhs.uk/Livewell
	Group 2 Reinforce previous aims. Content: review dietary goals & intake of cholesterol lowering foods in last week, examine barriers, deal with setbacks.	Recipe booklet produced in house. UCLP booklets with recipes (2012 Alpro)
Month 4		
Telephone follow up	As before	
Month 6		
Outcome Assessment	Group1	
	Group 2 Content: sustaining new dietary norms, where to buy foods now they are no longer supplied, how to obtain unusual food stuffs, achieving long term adherence to Mediterranean dietary pattern.	Nuts and their healthy smart fats (2013 Australian Tree Nut Industry) 'Lowering your cholesterol' tear off pad with voucher (2013 Flora)

Month 12		
One-year observations. Thanks for participation in study.	Group 1: Give information leaflets from Group 2 and offer cross over option where they will receive supermarket delivery and have fasting lipids measured at month 18.	

The BFF Trial protocol (version 3, 22 January 2015) prepared for NRES Committee West Midlands Solihull contains Standard Operating Procedures for each research visit and is available on request from author. For example, the Allocation visit reads:

- Collect Actigraph and food diary from participant.
- Give quality of life questionnaires, PE1 and Mediterranean Diet Score to participant to complete whilst waiting to see doctor.
- Undertake randomization procedure.
- When participant has seen doctor, walk to MIDRU for consultation with dietitian.
- Offer refreshments.
- Review food diary and seek clarification as required.
- Review PE1 questionnaire and discuss readiness to change question.
- Dietary consultation and advice, as appropriate for intervention group.
- Give written information and contact details for any queries participants may have during study.
- Provide one month supply of nuts and plant stanols to participants in group 2.
- Arrange mutually agreeable date and time in 1 month for next visit.

## Appendix 11. Strategy to deal with missing data.

Intention-to-treat analyses was planned, as stated in the protocol (Altman 1991), to be conducted with all participants included, in the groups to which they were randomised. This method creates treatment groups that do not differ systematically by any factors except the intervention assigned in the trial, thus avoiding selection bias. However, inclusion of all participants is rarely attainable in reality, due to missing data. Historically opinions have been divided on the best way to proceed, as no option is ideal. The choice is between omitting participants without final outcome data, or estimating the missing outcome data (Moher et al. 2001). Imputation, such as last value carried forward, was discussed during protocol development but decided against due to the potential to exaggerate the effectiveness of the intervention, and its reliance on the assumption of being conditional on covariates in the adjusted analysis. 'Complete (or available) case analysis' of observed data was preferred and is accepted by CONSORT (Moher et al. 2010) because participants who drop out cannot be included in the analysis. Further revision in 2010 suggested that reporting of the numbers included in the analyses and whether the analysis was by original assigned groups was essential rather than the strict definition of ITT analysis (White et al. 2011).

To resolve the issue of missing data a four-point ITT analysis-strategy was adopted (Altman 1991).

- 1) Attempts were made to follow-up all randomised individuals, even if they withdrew from allocated treatment.
- 2) The main analysis used all observed data and was valid under a plausible assumption about the missing data.
- 3) Sensitivity analysis was performed to explore the impact of departures from the assumption.
- 4) All randomised individuals were accounted for.

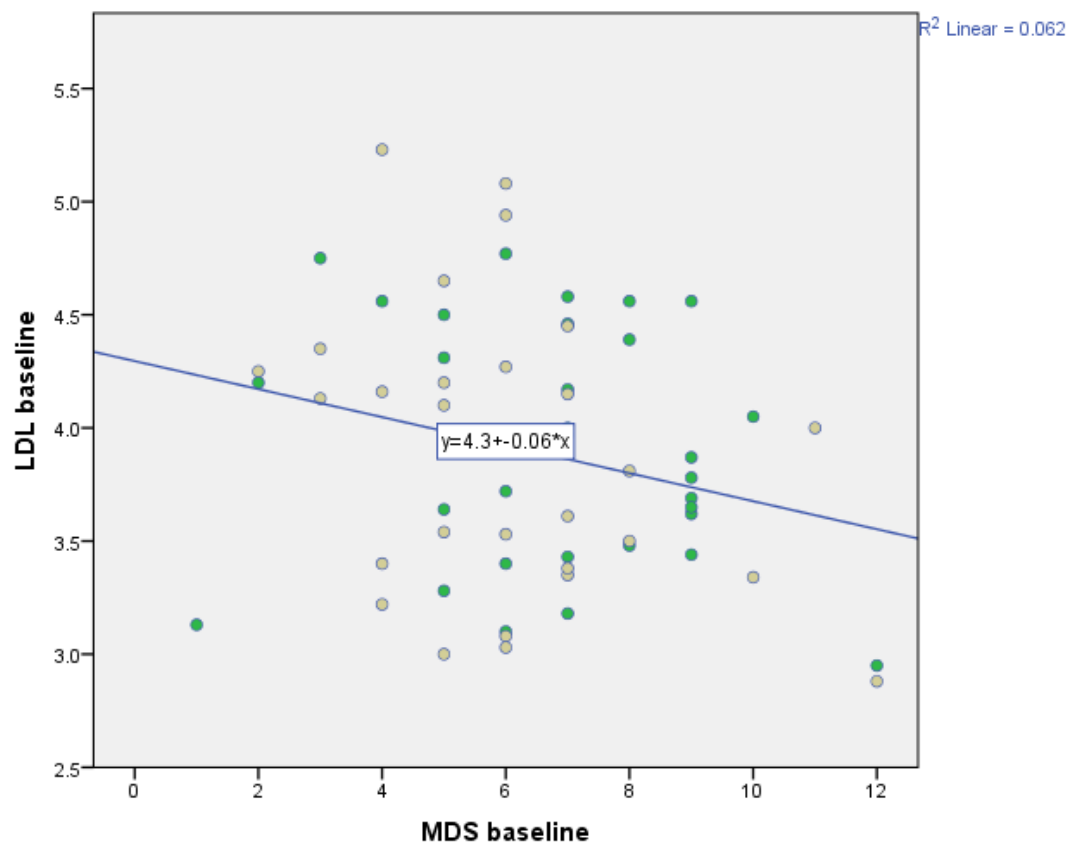
Regarding the first point, to enable inclusion of lost to follow-up participants in the analysis; medical records were reviewed to extract blood results, blood pressure, and weight from routine clinic attendance around the time of month 6. This enabled the intention to treat analysis to be conducted at month 6 for the primary outcome, for all participants according to their randomisation group, without the need for imputation of missing data.

Despite these efforts, there was missing data for 4 of the 7 participants who were lost to follow-up at month 12. To satisfy the second point, an 'available case' analysis was conducted for outcomes at month 12, acknowledging the limitations of the assumption that participants were missing at random. This is considered reasonable where number of LTFU is small, and proportion is similar in each treatment group (Martinez et al. 2012).

To explore the impact of the missing data, an intention to treat analysis was conducted with imputation of the baseline value where neither month 12 outcomes nor clinical data were available (for LDL-cholesterol  $n = 4$ ). This was undertaken as part of the sensitivity analysis for point 3. The rationale for assuming no change was determined from recent switch studies, where the control arm continued on their existing antiretroviral regimen of drugs and lipid levels remained relatively stable over a one-year follow-up period. For example, LDL-cholesterol increased 0.07mmol/l in SPIRAL (Pett et al. 2016) and 0.1mmol/l in MARCH (Llibre et al. 2018) on protease inhibitor regimens, and fell by 0.03mmol/l in SWORD (Mills et al. 2016) and 0.05mmol/l (Mills et al. 2016) on various regimens including NNRTIs. It is therefore plausible to assume that not following the dietary intervention would result in a negligible change in LDL-cholesterol such that the baseline value would be representative at month 12. This analysis was therefore only valid as an unadjusted analysis (Appendix)

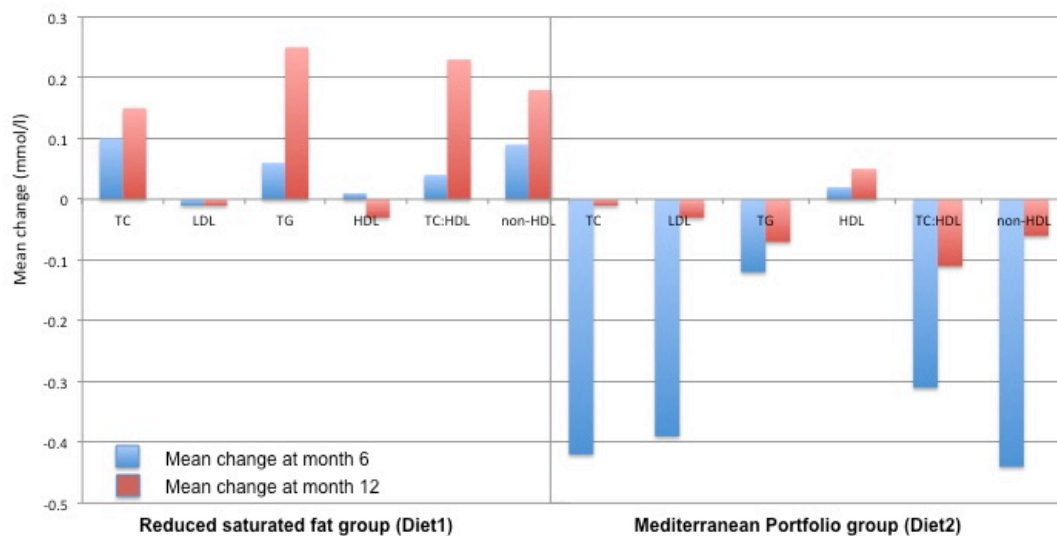
## Appendix 12. Relationship between Mediterranean Diet Score and LDL-cholesterol

Scatterplot using measures at baseline;  
(low saturated fat in green; Mediterranean Portfolio diet in yellow).



### Appendix 13. Mean change in lipid levels from baseline to month 6 and month 12.

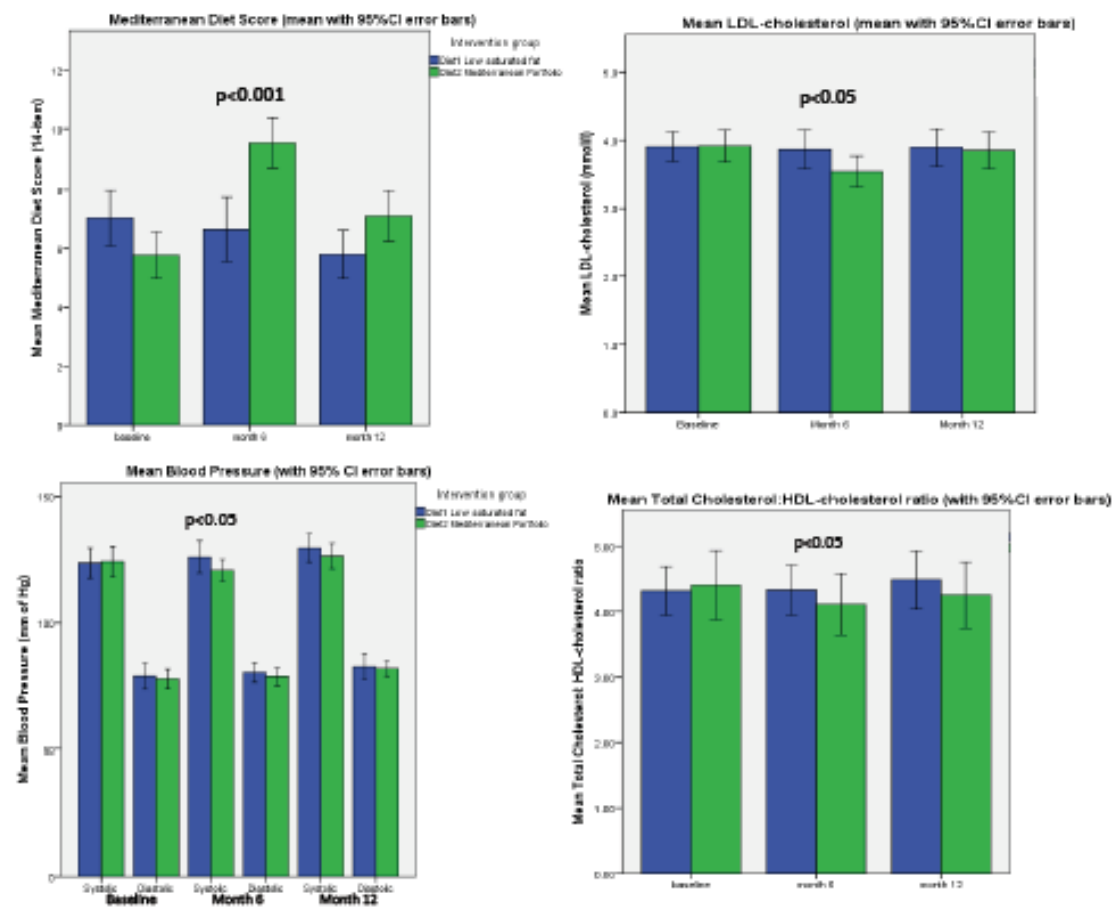
By group.



Whilst this figure illustrates the comprehensive beneficial changes to lipid profile in Diet2 group, there is no adjustment for baseline values in this data, therefore it displays regression to the mean rather than an accurate representation of the findings.

## Appendix 14. Cardiovascular risk factor measures at baseline, month 6 and month 12.

By intervention group.



## Appendix 15. Gut Function Questionnaire.

### GUT FUNCTION EVALUATION

**Participant**

**ID**

**Date:** \_\_\_\_\_

Do you currently have any problems with your stomach or bowels? **(Circle one)**

Yes

No

**Please rate your stomach or bowel symptoms during the last week by placing a tick in the box that best describes each symptom**

(Please tick the “none” box if you do not have this symptom)

	No symptoms or very rarely	Occasional or mild symptoms	Frequent symptoms that affect some social activities	Continuous symptoms that affect most social activities
	None	Mild	Moderate	Severe
Abdominal pain/discomfort	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Abdominal bloating/distension	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Increased flatulence/wind	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Belching or burping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Stomach/abdominal gurgling	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Urgency to open bowels	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	_____	_____	_____	_____



**Incomplete evacuation**

☐☐☐☐

**(feeling of inability to pass all stool)**

**Nausea**

☐☐☐☐

**Heartburn**

☐☐☐☐

**Acid regurgitation**

☐☐☐☐

**Tiredness/lethargy**

☐☐☐☐

**Overall symptoms**

☐☐☐☐

**1. Currently, how often do you pass a bowel action? (please tick one box)**

Once a week

☐

Once every 4-6 days

☐

Once every 2-3 days

☐

Once a day

☐

2-3 times a day

☐

4-6 times a day








☐

7 or more times a day

☐

Please tick the box that best describes your current stool:

## Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on its surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges (passed easily)
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. <b>Entirely Liquid</b>


2. Thinking about what you eat and drink at the moment, and any medicines you take, do any of these affect your stomach or bowels?

--

3. Any other comments about your stomach or bowels?

--

## Appendix 16. Sensitivity analysis for LDL-cholesterol

with imputation of missing values at month 12 with baseline value.

	Diet1 Low saturated fat		Diet2 Mediterranean Portfolio diet		Mean difference	P value
	Mean (SD)	n	Mean (SD)	N		
Baseline	3.91 (0.54)	31	3.88 (0.63)	29		
Month 12	3.90 (0.72)	28	3.85 (0.70)	28	-0.04 (-0.42 to 0.34)	0.8
Month 12 imputed	3.90 (0.69)	31	3.82 (0.71)	29	-0.08 (-0.44 to 0.28)	0.7

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