The Mediterranean diet and multiple sclerosis: a case-control study in The Republic of Cyprus.

A thesis submitted for the degree of Doctor of Medicine

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Statement of originality declaration

I hereby declare that the contents of this thesis are my own, have not been submitted or accepted in any previous application for a degree or other qualification, and are a true record of the work carried out by myself unless stated otherwise. I arranged the ethics approval in both the UK and Cyprus. I used a Food Frequency Questionnaire derived from The Epic-Norfolk questionnaire and questions from the Newcastle Thousand Families Study. The choice of questions was driven by the literature review. I arranged for two professional translations of the questions, one English to Greek and then back from Greek to English. I organized the printing and distributed the questionnaires myself to cases and controls throughout Cyprus. I collected the completed questionnaires and the data was entered into an excel file, which I uploaded into STATA 15.1. and I then analysed the results.

All quotations have been distinguished by quotation marks and all sources of information are referenced.

Paul Johnson August 2019

Abstract

Background: It has been suggested that dietary habits are associated with multiple sclerosis. Some eating patterns have been considered protective while others harmful.

Objectives: A case–control study was used to examine the association between multiple sclerosis and the Mediterranean diet, food groups and individual foods.

Methods: A total of 127 multiple sclerosis cases and 718 controls from across The Republic of Cyprus completed a self-reported questionnaire which included lifestyle and food questions. A 9-unit Mediterranean diet score (MDS) was calculated. Logistic regression was used to assess associations. An age-matched analysis of 119 cases and 119 controls was evaluated using conditional regression.

Results: In the logistic regression analysis adjusted for age, a higher MDS (OR=0.96, 95% CI: 0.93-1.00, p: 0.04) was inversely associated with being a multiple sclerosis case. Significant inverse associations were found in the following groups: vegetables (OR=0.83, 95% CI: 0.70-0.98, p: 0.03) alcohol (OR=0.87, 95% CI: 0.80-0.95, p: 0.001) legumes (OR=0.78, 95% CI: 0.64-0.95, p: 0.01) non-refined cereals (OR=0.81, 95% CI: 0.66-0.98, p: 0.03) while significant associations were found with dairy products (OR=1.24, 95% CI: 1.05-1.46, p: 0.01). Similar results were seen in the age-matched conditional regression analysis.

Conclusions: There was found to be a statistically significant association between a Mediterranean type diet and multiple sclerosis. There was an inverse association of being a multiple sclerosis case, with an increase in the MDS. Certain food groups and individual foods may also be associated with an increased or decreased association of being a multiple sclerosis case. This study may have significant effects on health planning in The Republic of Cyprus. When interpreting the results, there is always the possibility of reverse causality. That is, the disease itself, or the diagnosis of the disease, may lead to a change in eating habits. It is recommended that a Mediterranean type diet type be considered as a prevention measure to investigate in future intervention studies.

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I would like to thank all the people who have helped and supported me over the last three years in the preparation of this MD thesis, especially the people of The Republic of Cyprus who gave their time to fill in the questionnaire.

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Table of Contents

Table of Figures

Chapter 1. Introduction

1.1 The Study

This case-control study in The Republic of Cyprus, compares Mediterranean diet adherence between people with multiple sclerosis (the cases) and people without multiple sclerosis (the control participants). The rationale for the work is that very few studies exist on the subject and none have been performed in Cyprus or Europe before.

1.1.1 *An introduction to The Mediterranean Diet*

The Mediterranean diet is not one specific diet. It has existed primarily in the Mediterranean basin area for centuries. It is regarded as a rather frugal eating pattern with high consumption of fruits, vegetables, whole grains, nuts and legumes. Meat consumption, especially red meat and processed meat products is low (Devries Van Horn and Willett 2018). Since the early 1960s, the health benefits of the Mediterranean diet have been widely documented (Keys 1995) and "robust evidence" is said to exist for health benefits of Mediterranean diet adherence in cardiovascular diseases, neurodegenerative diseases and diabetes (Dinu et al. 2017). The literature review in Chapter 2 will discuss in detail The Mediterranean diet and potential health benefits. The role of diet in susceptibility to disease including multiple sclerosis is also dealt with in detail in Chapter 2.

1.1.2 *Why study multiple sclerosis?*

Multiple sclerosis is a chronic progressive, debilitating disorder of the central nervous system, which is probably caused by genetic susceptibility combined with an environmental trigger or triggers (Wu and Alvarez 2011). It is an interesting disease for study as there remain many unanswered questions. Few case-controls studies involving the Mediterranean diet and multiple sclerosis have been published. The Mediterranean diet is a lifestyle choice that has been advocated for other chronic disease prevention, but insufficient scientific evidence is currently available to recommend it in multiple sclerosis prevention.

A more detailed description of multiple sclerosis can be found in a later section of the introduction.

1.1.3 *Why Cyprus?*

Cyprus is an island situated at a latitude of 35.1264° N, and a longitude of 33.4299° E. Latitude or more accurately, distance from the equator, has been regarded as a risk factor to acquiring multiple sclerosis (Sundström and Salzer 2013). Other countries approximately on the same latitude and within 2000 Km include Crete, Malta, Tunisia, Syria, Iraq and Iran. It might be expected that Cyprus would have a similar prevalence of multiple sclerosis to the countries on the same latitude and a lower prevalence than some more northern countries in Europe. Unfortunately, Cyprus does not have a data base recording all multiple sclerosis cases yet, and the prevalence is therefore unknown. Anecdotal evidence from current hospital appointments in Cyprus suggests that the incidence of multiple sclerosis cases per 100,000 population is higher than countries on the same latitude (Charalambidou Pantzaris and Patrikios 2016). This begs the question why? Although a definitive answer cannot be given, it is possible that demographics and lifestyle are different between Cyprus and other countries on the same latitude. The prevalence section of this introduction will deal with this in more detail.

A brief overview of Cyprus

Cyprus is the third largest island in the Mediterranean Sea at around 9,251 square kilometers. It is 225 kilometers (km) long at its longest point and 96.5 km at its widest point (The World Factbook 2016-17). The island is situated in the Eastern Mediterranean Sea with its nearest neighbours being Turkey, which lies approximately 113 km to the north and Syria which lies 120 km to the east. Lebanon and Israel also lie to the east being 264 km and 472 km east respectively. To the west, on approximately the same latitude, Crete is 785 km away and Rhodes, to the north west, is the nearest of the Greek islands (492 km) while Egypt is the nearest country to the south of Cyprus at approximately 954 km (The World Factbook 2016-17). The island was effectively divided in 1974 after an invasion by Turkey and a UN buffer zone remains unoccupied and divides the island into Greek and Turkish territories including the city of Nicosia. Gathering research material in the occupied area had significant challenges, so this research was confined to The Republic of Cyprus and from this point on, this region will be called Cyprus.

Cyprus has four major cities and 5 districts as shown in Figure 1. Nicosia is the capital and the largest city. It has two public hospitals, Nicosia General Hospital and Archbishop Makarios lll Hospital, which is the main hospital for children and maternity treatment. Most neurology, including consultations for patients with multiple sclerosis occurs at The

Cyprus Institute of Neurology and Genetics (CING) which is a public institution in Nicosia. Limassol, Larnaca and Paphos have public general hospitals, but multiple sclerosis patients are generally referred to CING in Nicosia. Cyprus has numerous private hospitals and many small private clinics but multiple sclerosis patients attending these private centres can get free prescriptions via the dispensary at CING, so they also attend there.

Figure 1 The cities and districts of The Republic of Cyprus (The University of Central Arkansas 2018)

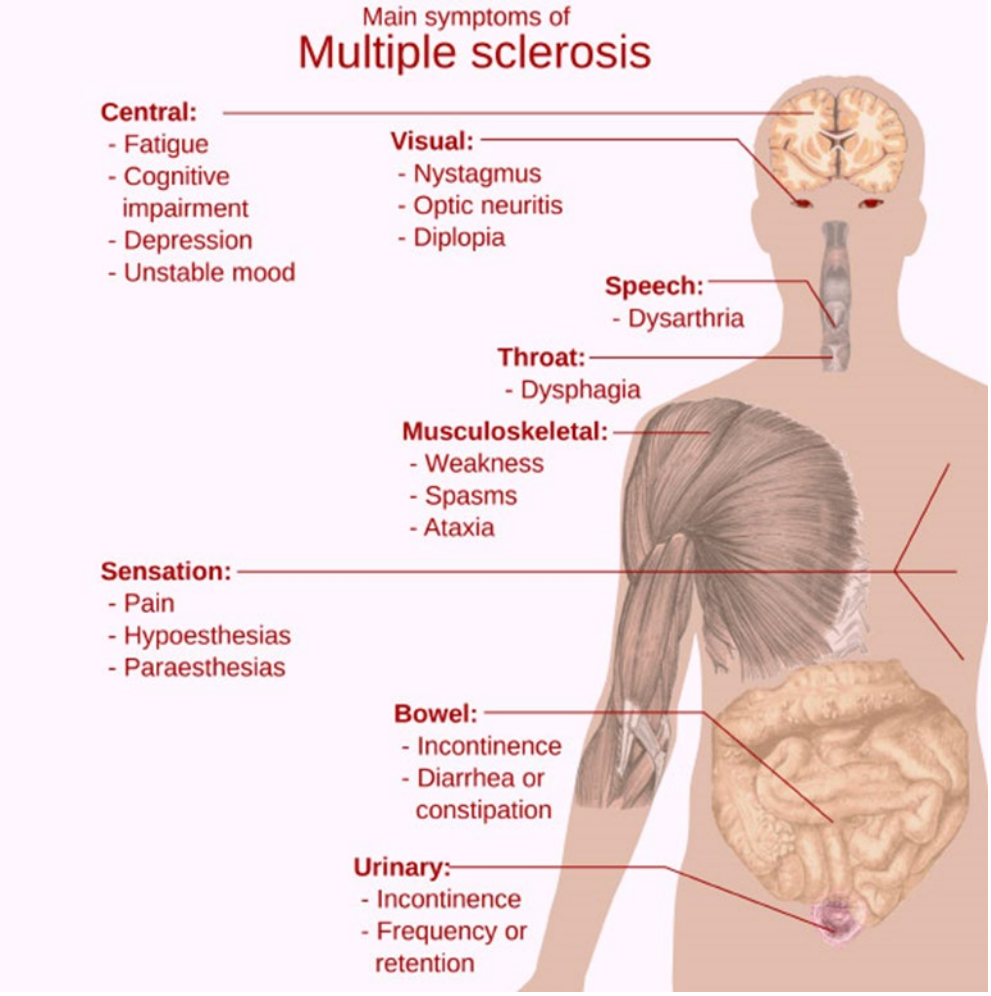
The most recent estimate by The Republic of Cyprus Statistical Service (2017) is that at the end of 2016 there were 706,800 Greek Cypriots (74,6%) 92,200 Turkish Cypriots and 148,000 Foreign residents on the island (excluding illegal settlers from Turkey and elsewhere). The total population of the Government controlled area was estimated at 854,800. Tertiary education attainment in Cyprus, is the 2nd highest in the EU and is higher amongst women than men. About 38% of women aged 25 and over have completed tertiary education and by 34 years of age 63.5% of women and 47.2% of men have successfully completed tertiary education. Around 58% of women aged 15 years and over are in the labour force, and 66% of men. Monthly earnings are around €1,600 for women and €2,100 for men. Cypriots are among the most prosperous people in the Mediterranean region with nominal GDP per capita exceeding $28,000 in 2018 (IMF 2019). Despite this, around 30% of women are said to be at risk of poverty or social exclusion. The life expectancy of woman in Cyprus at birth is 83.5 years compared with 79.8 years for men (The Republic of Cyprus Statistical Service 2017). Socioeconomic

status has been suggested as a risk factor for multiple sclerosis and this is discussed in detail later.

1.2 Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic, mostly progressive, inflammatory disease of the nervous system, which mainly affects young and middle-aged adults (Rice et al. 2001; Dendrou et al. 2015). It is the most common autoimmune disorder affecting the central nervous system and is about three times as common in women as men, although the sex ratio has shifted over the years and this will be described in more detail in chapter 2 (Golden and Voskuhl 2017; Waubant 2018). Serious disability such as, bowel and urinary incontinence, cognitive impairment and difficulty walking (Kister et al. 2013) can result from repeated damage to the myelin sheaths of nerves (Jagannath et al. 2010). In simplistic terms, it is thought that in genetically susceptible people, an environmental trigger leads to a complex cascade of events, ultimately resulting in lymphocytes targeting oligodendrocytes and myelin. This can cause a number of signs and symptoms. The main symptoms are shown in Figure 2.

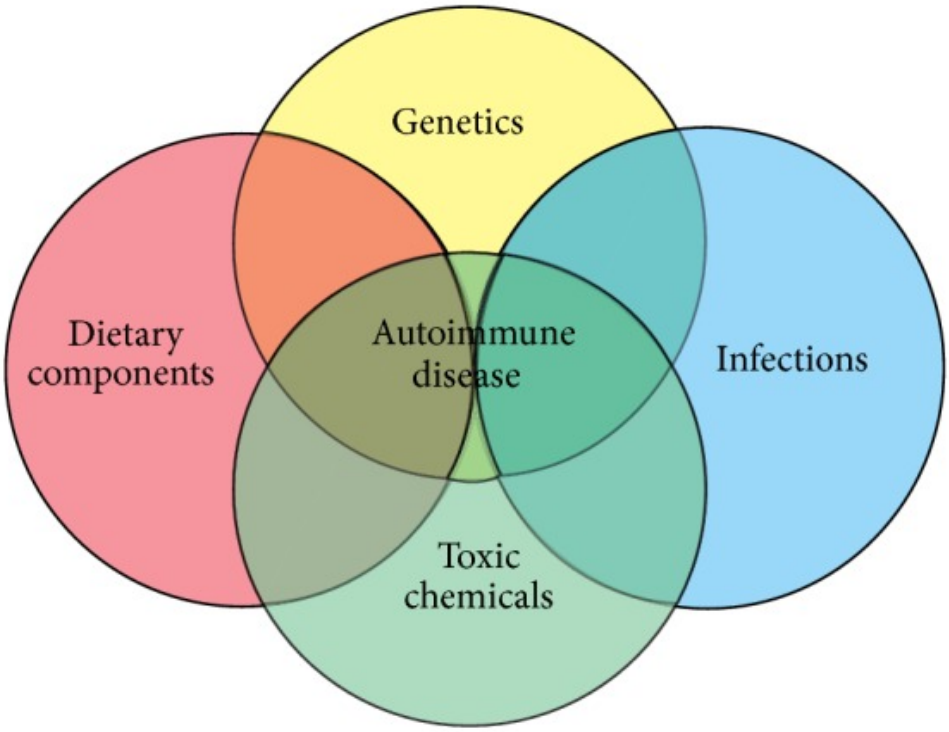
Figure 2 The main symptoms of Multiple sclerosis (Häggström 2014)



1.2.1 *Environmental determinants of autoimmunity*

Genetic predisposition is thought to account for about 30% of all autoimmune diseases while 70% is due to environmental factors (Vojdani Pollard and Campbell 2014). The main environmental elements are summarised in Figure 3.

Figure 3 The interplay of genetics and environmental factors in autoimmunity (Vojdani Pollard and Campbell 2014).



According to the American Autoimmune Association (2018) over 100 autoimmune diseases have been identified. These diseases may be organ specific or systemic. Well known examples include multiple sclerosis, type 1 diabetes and rheumatoid arthritis. The prevalence of these diseases is higher in developed countries and the incidence is increasing (Chowaniec Kawalec and Pawlas 2017). It is thought this is most likely due to exposure to environmental factors. The environmental factors thought to influence acquiring multiple sclerosis are dealt with in detail in the literature review and the immunopathology of multiple sclerosis will be discussed later in this chapter.

1.2.2 *A brief history of multiple sclerosis*

In 1395, a 16year-old girl from Schiedam in Holland developed an illness and fell while skating on a frozen canal. She later developed blindness in one eye, weakness and pain. She died in 1433. She was canonised by the church and became the patron saint of figure skating and sickness. She was named Lidwina The Virgin. It has been suggested by Orrell (2005) that this was the first recorded case of multiple sclerosis. Augustus d'Este (1794–1848), who was the grandson of George III of England, is also said to have had of multiple sclerosis (Murray 2004). He exhibited transient blindness at the age of 28 years as the first symptom. He documented his illness in a diary for the next 44 years. He eventually could not walk and used a wheelchair before being confined to bed. He died aged 54 in1848 (Murray 2004).

In 1868 the French neurologist Jean-Martin Charcot described the disease and the phrase "Charcot's triad" was coined. It refers to intention tremor, nystagmus, and scanning speech which are common in multiple sclerosis. He suggested these clinical signs were associated with the destruction of myelin (Talley 2005).

1.2.3 *The diagnosis of multiple sclerosis*

The diagnosis of multiple sclerosis is a combination of clinical and magnetic resonance data, with cerebrospinal fluid (CSF) analysis and other paraclinical tests sometimes being used. There are no pathognomonic tests (Pugliatti et al. 2006). The diagnostic critera have evolved with advances in technology, particularly magnetic resonance imaging (MRI) (Poser and Brinar 2004). The Schumacher et al. criteria (Kurtzke 1993) were the first to be internationally recognised criteria for diagnosis in 1965 (Poser and Brinar 2004). The McAlpine, Lumsden, and Acheson criteria were also suggested in 1965 and the Poser et al. criteria published in 1983 (Poser and Brinar 2004). The McDonald Diagnostic Criteria were adopted in 2001 (Poser and Brinar 2004), revised in 2005 and again 2010 (Polman et al. 2011). In 2017, a revision was made to the 2010 McDonald Diagnostic Criteria by the International Panel on Diagnosis of Multiple Sclerosis (Thompson et al. 2018). The diagnostic criteria have been simplified and are shown in Figure 4.

Figure 4 The 2017 update to 2010 McDonald Diagnostic Criteria (Thompson et al. 2018).

Clinical Presentation	Additional Data Needed for Diagnosis
≥ 2 clinical attacks and objective evidence of ≥ 2 lesions	None
≥ 2 clinical attacks and objective evidence of 1 lesion	DIS: an additional attack implicating a different CNS site OR by MRI[a]
1 clinical attack and objective clinical evidence of ≥ 2 lesions	DIT: an additional clinical attack OR by MRI[b] **OR** CSF-specific oligoclonal bands
1 clinical attack and objective evidence of 1 lesion	DIS: an additional clinical attack implicating a different CNS site OR by MRI[a] **OR** DIT: an additional clinical attack OR by MRI[b] **OR** CSF-specific oligoclonal bands

Adapted from Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* 2018;17:162–73.
Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; DIS, disseminated in space; DIT, disseminated in time; MRI, magnetic resonance imaging.
[a]DIS by MRI: new lesions on follow-up imaging or both gadolinium-enhancing and non-enhancing lesions on single MRI.
[b]DIS by MRI: ≥ 1 symptomatic or asymptomatic lesion in ≥ 2 areas including cortical/juxtacortical, periventricular, infratentorial, or spinal.

Dissemination in Space (DIS) can be demonstrated when lesions on T2 MRI can be seen in at least two out of four areas of the CNS: periventricular, juxtacortical, infratentorial, or spinal cord. Dissemination in Time (DIT) requires a new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI or the simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time.
The clinical course of multiple sclerosis is characterized by relapses and/or disease progression which will be explained in the section on clinical phenotypes.

1.2.4 *Clinical Phenotypes*

The disease course varies from patient to patient, but a number of clinical phenotypes have been described over the years for the purposes of communication and management. In 1996 the US National Multiple Sclerosis Society defined the clinical subtypes in order to standardize the terminology (Lublin and Reingold 1996).

Four categories of phenotype are classically described in the literature based on the course of disease (Roosendaal and Barkhof 2015).

1. Relapsing–remitting multiple sclerosis is the most common form. About 85% of multiple sclerosis patients are affected. The disease typically shows relapses or flare ups of symptoms followed by periods when symptoms improve or disappear (remission).
2. Secondary progressive multiple sclerosis. This may develop in some patients with relapsing–remitting disease when the disease continues to worsen with or without periods of remission or leveling off of symptom severity (plateaus).
3. Primary progressive multiple sclerosis affects approximately 10% of multiple sclerosis patients. Symptoms gradually worsen from the beginning without relapses or remissions.
4. Progressive-relapsing multiple sclerosis is rare and affects less than 5% of patients. It is progressive from the start, with no periods of remission, but there may be intermittent flare-ups of worsening symptoms at times.

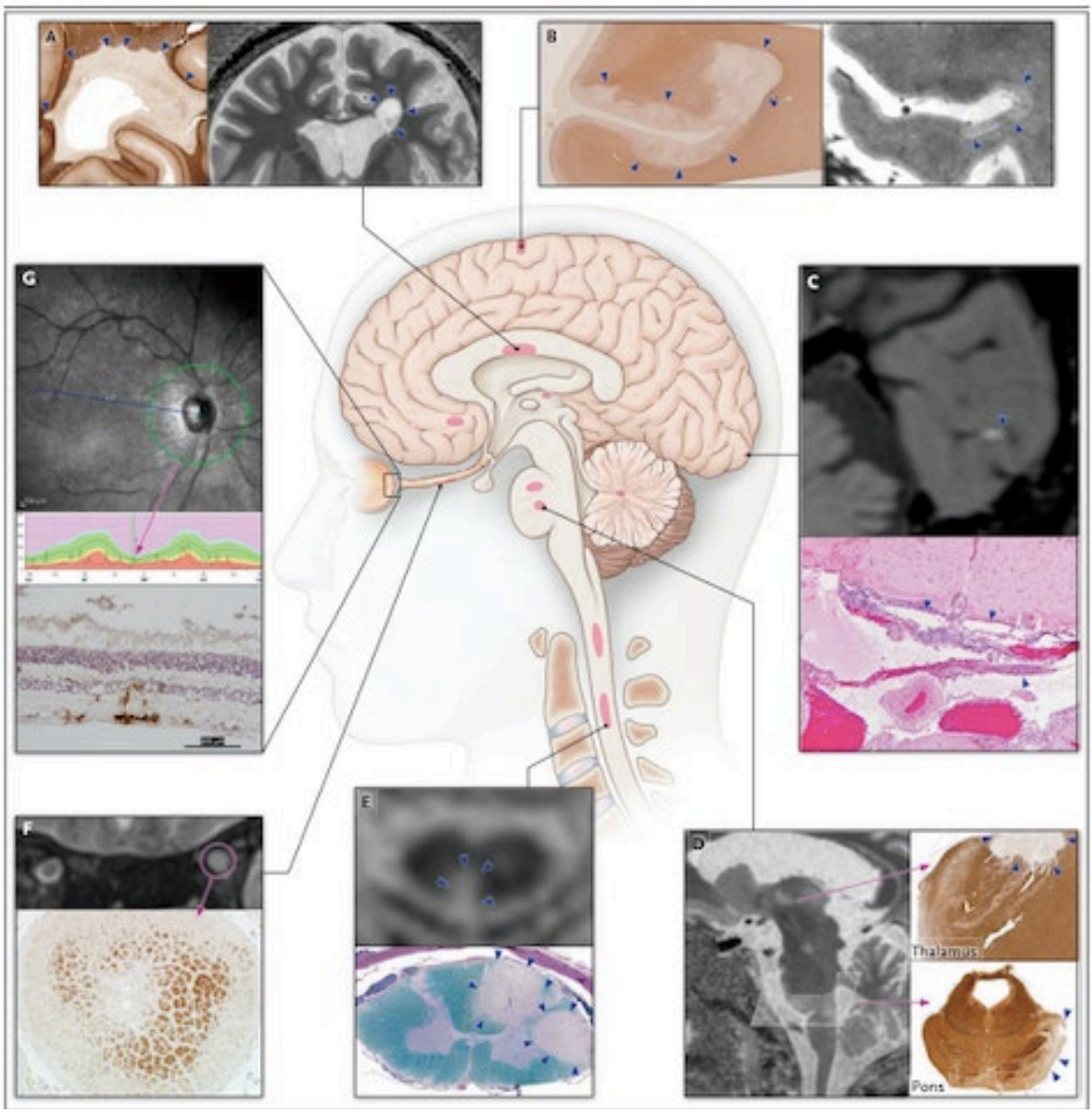
The first demyelinating event which is labelled "clinically isolated syndrome" (CIS) may prompt suspicion of multiple sclerosis, but until the patient fulfils the McDonald Diagnostic Criteria (revised in 2017) the condition cannot be called multiple sclerosis (Direnzo et al. 2015). In one study between 30% and 40% of patients showing CIS symptoms progressed to a diagnosis of multiple sclerosis within one year and 60% within three years (Wottschel et al. 2014). However, 20 % of CIS patients do not convert to multiple sclerosis within two decades. Briggs et al. (2019) found that obesity and smoking were two risk factors that modulated different phenotypes but in the current study all phenotypes were recruited as cases so long as they met the selection criteria. In part, this was due to the small numbers of phenotypes 2,3 and 4 and also the general slowness in case recruitment.

1.2.5 *Simplified Pathophysiology and immunopathology of multiple sclerosis.*

Introduction

Multiple sclerosis is a complex neurodegenerative disease of the central nervous system which is considered to be autoimmune (Huang Chen and Zhang 2017). It is a dissemination of plaques and neurological changes found throughout the central nervous system including white matter, gray matter, brain stem, spinal cord, and optic nerves (Longo et al. 2018) as seen in Figure 5.

Figure 5 The topography of multiple sclerosis lesions (Longo et al. 2018).



A denotes the periventricular region, B the cortex, C the leptomeninges, D the thalamus and pons, E the spinal cord, F the optic nerve, G the retina. MRI findings from different patients are shown along with histology results at post-mortem.

The myelin sheath in the central nervous system is an insulating layer of protein and fat made by oligodendrocytes and acts to allow fast and efficient transmission of impulses along the nerve axon. Inflammation leads to demyelination and damaged nerves which cannot transmit the nerve impulse either at all or as effectively as before (Frohman Racke and Raine 2006).

Inflammation of the central nervous system (CNS), demyelination, axonal injury and eventually axonal loss are characteristic of multiple sclerosis, but the antigen or antigens

in this immune-mediated response have yet to be found (Kamm Uitdehaag and Polman 2014). In the early stages of relapsing-remitting multiple sclerosis, there is focal inflammation resulting in white matter plaques. These are areas of demyelination and they may be accompanied by varying degrees of axon loss and subsequent reactive gliosis with the production of hypertrophic astrocytes. Typical areas of pathology seen on MRI are found in the periventricular, juxtacortical and infratentorial areas of the brain, or in the spinal cord (Thompson et al. 2018).

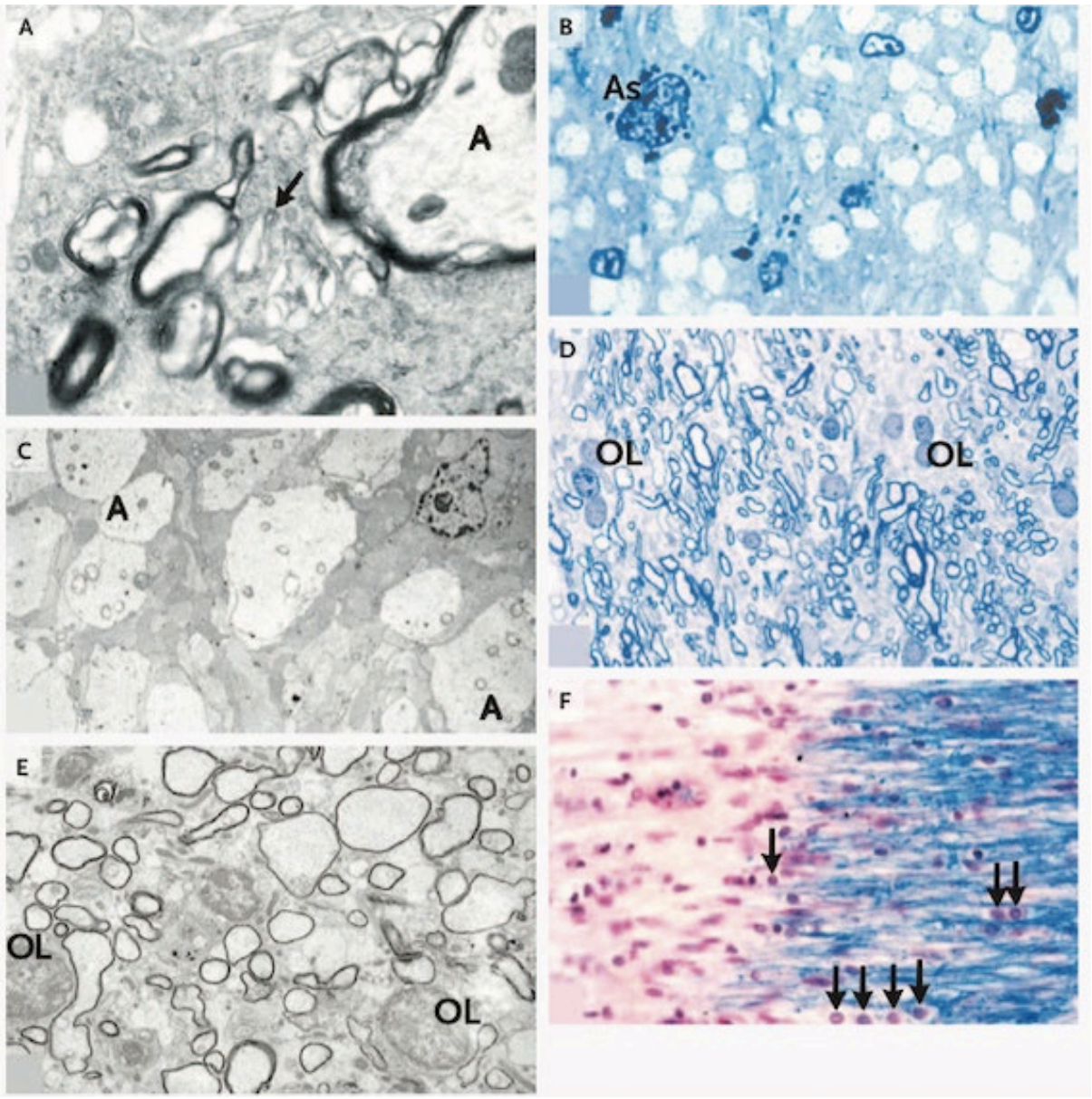
Histopathology

While focal white matter lesions are characteristic of histopathology seen at post-mortem and are characterized by demyelination and inflammation, gray matter demyelination varies considerably between individuals. Infiltration of immune cells, including T cells, B cells and myeloid cells, into the central nervous system are thought to cause injury and form the focal lesions (Filippi et al. 2018).

The histology of a chronic lesion is shown in Figure 6.

Panel A shows demyelination of a myelinated fibre.
Panel B shows astrocytes (AS) and astroglial scar tissue
Panel C shows demyelinated axons(A) within the glial scar
Panel D shows an area of remyelination with overabundant oligodendrocytes (OL)
Panel E shows remyelination but with thin myelin sheaths
Panel F shows there is an abrupt boundary between myelinated fibres (blue) and demyelinated plaque lesion. Oligodendrocytes are indicated with arrows and do not enter the lesion.

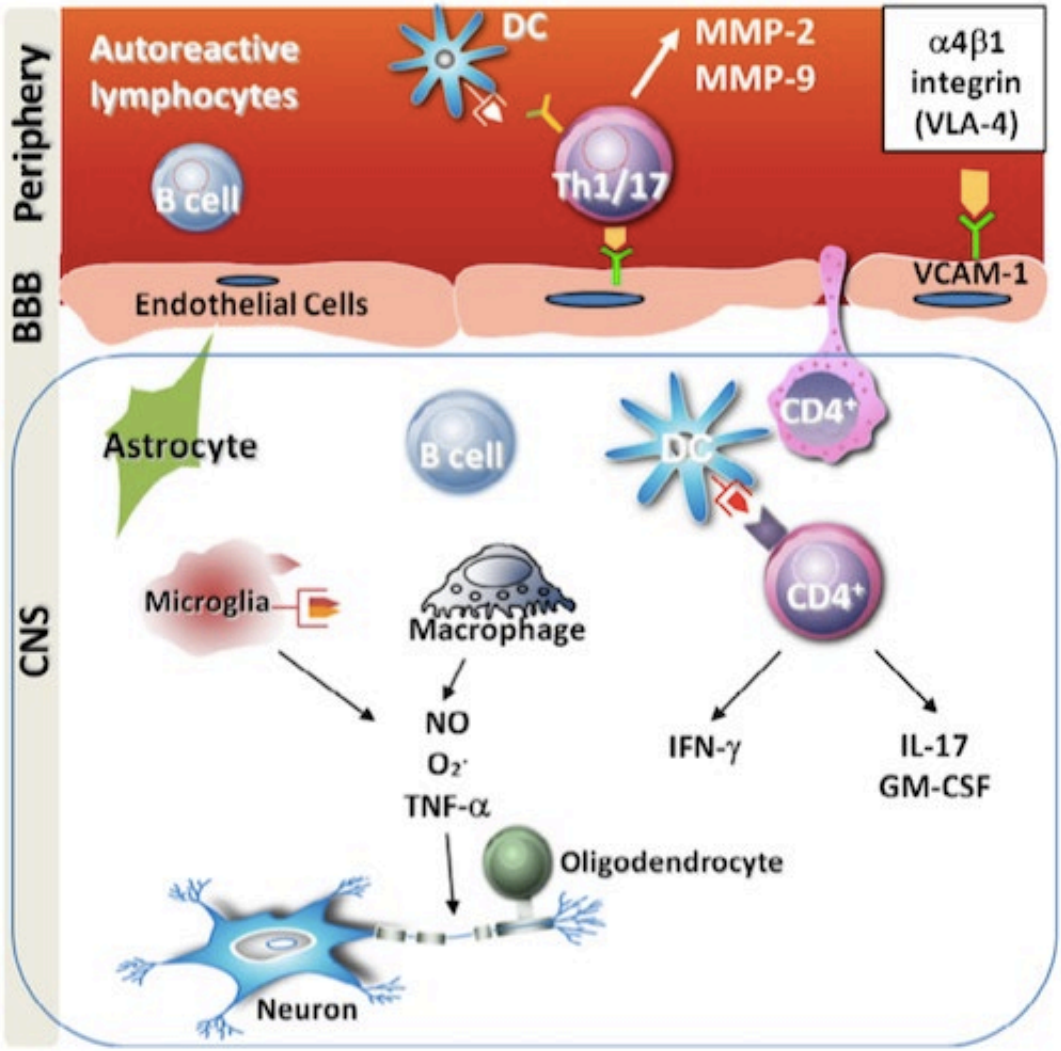
Figure 6 The histology of a chronic multiple sclerosis lesion (Frohman et al. 2006).



Immunopathology

As mentioned earlier, in genetically predisposed people, an environmental trigger or triggers is thought to activate a complex immune response. The immune system mistakes a patient's tissue as foreign and attacks it via a cascade reaction. In simplistic terms, this is the basis of autoimmunity. A possible group of cellular components interact in multiple sclerosis as shown in Figure 7.

Figure 7 A schematic representation of key cellular players in the autoimmunity of multiple sclerosis (Grigoriadis and Van Pesch 2015).



At some point in the pathogenesis the blood brain barrier is breached, and it seems likely that immune dysregulation leads to perivenular inflammatory lesions with demyelinating plaques. This "perivascular cuffing" a term used to describe the immune cell influx that forms around vessels is mainly composed of T lymphocytes, monocytes and macrophages (Wu and Alvarez 2011) but B-cells and plasma cells are also present. Foamy macrophages are involved in the stripping of myelin and there is a varying degree of oligodendrocyte apoptosis. Axons are initially preserved, but with disease progression these too are lost or badly damaged (Dobson and Giovannoni 2018).

1.2.6 *Epidemiology and suggested Aetiology of multiple sclerosis*

Introduction

It is often said that the aetiology of multiple sclerosis is unknown, but substantial evidence exists to support associations of various factors in developing the disease. Over 40 factors have been proposed as risks for acquiring multiple sclerosis with some risks having more evidence than others to support their role. In a genetically susceptible person, infection with the Epstein–Barr virus, low vitamin D concentrations and a lack of sun exposure, smoking and adolescent obesity have been well established as individual associations with increased risk for acquiring multiple sclerosis (Olsson Barcellos and Alfredsson 2017). The literature review deals with possible aetiology in detail in the next chapter. Kockum Alfredsson and Olsson (2014) state that along with at least 50 genetic polymorphisms, groups of risk factors have been identified that are jointly involved in the pathogenesis. Migration studies have supported the view that multiple sclerosis is due more to environmental exposure than genetics (Kurtzke 2013). However, genetics account for between 15-30% (Wang et al. 2016), as when compared to the general population. In the general population there is a risk of 0.1%, people with an affected first-degree relative have a 2 to 4% risk of multiple sclerosis (Salroo et al 2016). While concordance in monozygotic twins is between 30 and 50% (Longo et al. 2018). While the consensus view is that environmental exposure amounts to a large part of the aetiology, Ebers (2013) argues that there is no particular value in trying to estimate the relative importance of either, since the risk requires interaction between both elements. The disease affects about 3 times more women than men and the sex ratio has changed over the years (Waubant 2018), and women are diagnosed usually 2-5 years earlier than men. The literature review deals with this in more detail. Incidence declines with age in both sexes and initial diagnosis is less common in those over 60 years (Polliack Barak and Achiron 2001). Life expectancy in multiple sclerosis patients is reduced by 7-10 years compared with people without the disease (Kamm et al. 2014). It is a debilitating disease with significant impact on the patient, their family and society

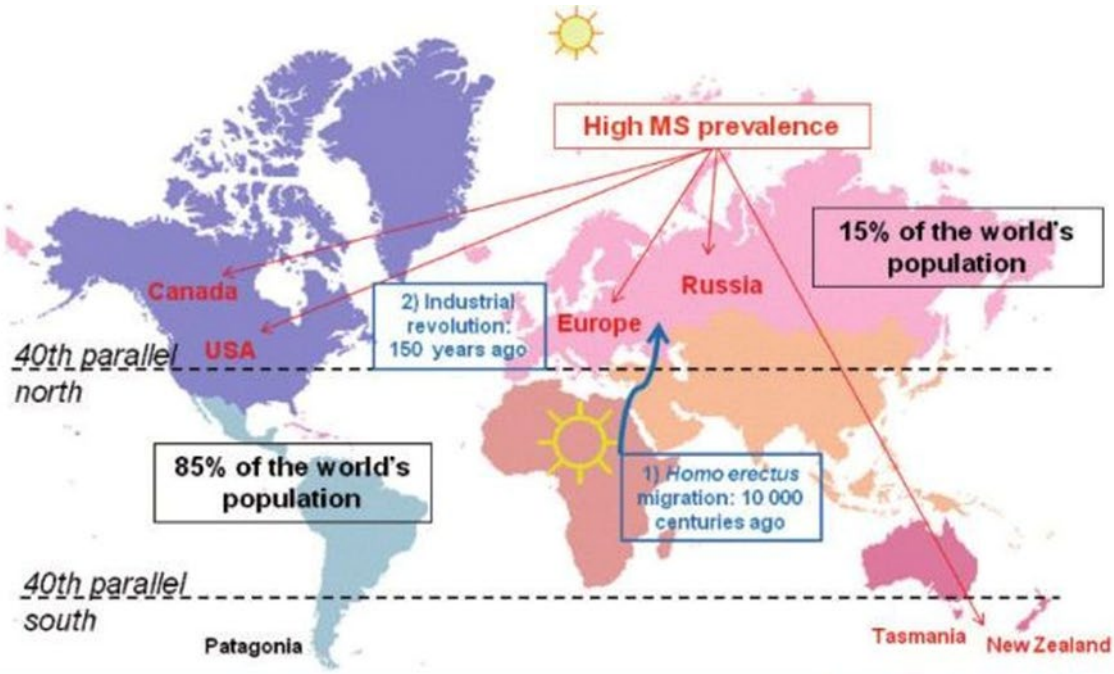
Prevalence and incidence

The prevalence of multiple sclerosis is the number of patients with multiple sclerosis alive on a specific date per 100 000 of a population, whereas the incidence is the number of new cases of patients with multiple sclerosis per 100,000 over a given period of time (a year is often used for multiple sclerosis). The disease is rare in children and the adult

prevalence peaks at 50 years of age, whereas the incidence for multiple sclerosis peaks at approximately 30 years of age (O'Gorman 2012). Incidence peaks close to the average age of diagnosis, while prevalence peaks much later and includes later diagnosed cases and is also influenced by survival. Wallin et al. (2019) suggest improved survival has resulted in a rising prevalence in the last fifty years, particularly in high income countries. Several countries in the world have registers of multiple sclerosis cases (Flachenecker et al. 2014). For example, Denmark has aimed to register all of its multiple sclerosis patients in a data bank. This allows prevalence and incidence, disease progression and treatment to be accurately recorded and monitored. In other countries registries have been set up where individuals volunteer to join but these data cannot be used to assess prevalence and incidence, since the data are incomplete. These registries rely totally on volunteer information.

While it is estimated there are over 2 million people with multiple sclerosis worldwide (Huang Chen and Zhang 2017), the prevalence is low in the tropics and increases as one moves north or south of the tropics as can be seen in Figure 8.

Figure 8 Map of the World showing areas of high multiple sclerosis prevalence and areas of low prevalence (Pierrot-Deseilligny and Souberbielle 2010).



Meta-analysis has confirmed the long-held view that a latitude gradient of multiple sclerosis incidence in Europe exists (Alcalde-Cabero et al. 2013; Kingwell et al. 2013; Sundström and Salzer 2013; Wang Simpson and Taylor 2018), although it is possible this

may reflect vitamin D levels which are affected by seasonal changes in sunlight exposure (Longo et al. 2018). This will be discussed later in the literature review. Other studies have suggested this latitude gradient has declined for example in Norway where Grytten Torkildsen and Myhr (2015) suggest there is no longer evidence of a latitude gradient. This may be due to people being exposed to similar environmental risk factors independent of geography. One possibility is that any vitamin D deficiency induced by latitude, has been addressed with vitamin D tablets, although there is no strong evidence to support this. In the UK several studies have attempted to provide up-to-date information on multiple sclerosis prevalence, and the UK remains one of the most studied areas in the world for multiple sclerosis prevalence (Kingwell et al. 2013). By 2012 there had been 28 unique prevalence or incidence studies of which 13 were from England, 6 from Scotland and 3 from Wales and 3 from Northern Ireland and 1 from Guernsey (Kingwell et al. 2013). In a descriptive study using data from the UK General Practice Research Database (GPRD) Mackenzie et al. (2014) suggest that the prevalence of multiple sclerosis in the UK (as at 2010) was 200 per 100,000 and there was an increase in prevalence when moving from the south to the north. The prevalence rate of 187 per 100 000 in south-east Scotland in 1995 was more than twice that for England and Wales at that time (Pugliatti et al. 2006). Robust evidence and meta-analyses tend to support this gradient. Wallin et al. (2019) suggest an increase in prevalence of 1·03 times per degree of latitude in the US. It is possible the south to the north increase in prevalence (in the northern hemisphere) could be an artefact. This might due to better case finding and/or better survival.

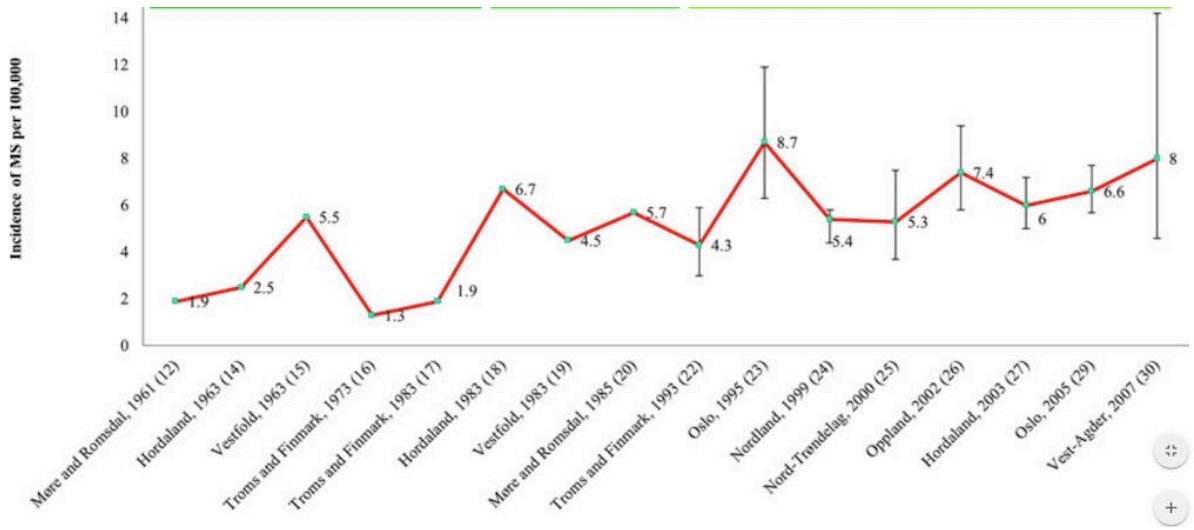
Alternatively, a true gradient of prevalence might exist due to prevalence differences. Carod Artal (2019) carried out a cross sectional observational study using data from the Raigmore Hospital register in the Scottish Highlands to establish the prevalence of multiple sclerosis in this population of around 250,000 people between 1 January 2016 to 31 December 2016. He found 745 patients met the inclusion criteria. Of these, 75.4% (562 cases) were female. He estimated the crude prevalence rate for multiple sclerosis in this population (the Highlands) was 300 cases per 100,000 inhabitants (95% CI: 280 to 320) and the mean age at diagnosis was 45.45 ± 13.27 years [range: 14-84 years]. This study provides the most up-to-date prevalence of multiple sclerosis available for this part of Scotland. Other UK prevalence data, for example that quoted by The Multiple Sclerosis Society (2019) uses 5-year-old data and applies a 2.4% increase per year to achieve an estimated figure.

The shifting incidence of multiple sclerosis

The incidence of multiple sclerosis appears to be increasing throughout the world in both developed and developing countries with increased smoking suggested as being a partial reason for the increase in incidence among women (Dobson and Giovannoni 2018). While smoking is decreasing in many high-income countries, multiple sclerosis appears still to be rising, suggesting factors other than smoking are involved.

Grytten Torkildsen and Myhr (2015) reported that the incidence of multiple sclerosis rose from 1.9 to 8 per 100,000 between 1961 and 2007 in Norway. The results are represented graphically in Figure 9.

Figure 9 Graph of multiple sclerosis incidence against time in Norway (Grytten Torkildsen and Myhr 2015).



In the Scottish Highlands, the data from Carod Artal (2019) show that there is a general trend of increasing incidence over even a short period of seven years as seen in Table 1.

Table 1 The incidence of multiple sclerosis in the Scottish Highlands between 2010 and 2016 (Carod Artal 2019).

Year of Diagnosis	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
Incident cases	36	23	30	39	45	41	47

The reason for this shifting incidence is uncertain, but it can be speculated that it is due to one or more of the following factors:

1. A true increase in multiple sclerosis incidence
2. An increase in early diagnosis of multiple sclerosis
3. A better multiple sclerosis service with easier access to MRI facilities
4. An increase in the number of multiple sclerosis specialist doctors and nurses, since more specialists might increase access to services and so increase diagnosis. Alternatively, an increase in multiple sclerosis incidence might increase the need for more multiple sclerosis specialist doctors and nurses.

1.2.7 *Methods of measuring new lesions in multiple sclerosis*

In addition to aiding diagnosis, MRI is being used to assess response to treatment and disease progression. New lesions (plaques and scars) found on MRI are usually but not always associated with disease symptoms (Kaunzner and Gauthier 2017). While specific imaging protocols are not suggested in the McDonald Criteria, evidence-based guidelines

were produced by The Magnetic Resonance Imaging in MS (MAGNIMS) network (2015).

A meta-analysis by Sormani and Bruzzi (2013) found an association between lesion development and relapses and Ontaneda and Fox (2017) suggest that with every clinical relapse, between 10 and 15 new lesions are formed. When new lesions are detected on MRI, it is possible to use MRI scans as a screening tool for disease activity or as a measure of therapeutic medication efficacy.

1.2.8 *Relapse and Remission in multiple sclerosis*

Relapses are defined as neurological symptoms that last for more than 24 hours in the absence of fever or infections while remissions are times in which symptoms may partially or completely subside. Remissions may be short periods of days or weeks, or longer periods of months or years and may be characterized by a return to a level of health similar or equal to that experienced prior to the last relapse (Pappalardo and Hafler 2019). Steinman (20209) suggests there are three main molecules involved in the process of relapses and remissions. These are α4β1 integrin and osteopontin which lead to the production of pro-inflammatory cytokines and αB crystallin which inhibits inflammation.

1.2.9 *Multiple sclerosis and the microbiome*

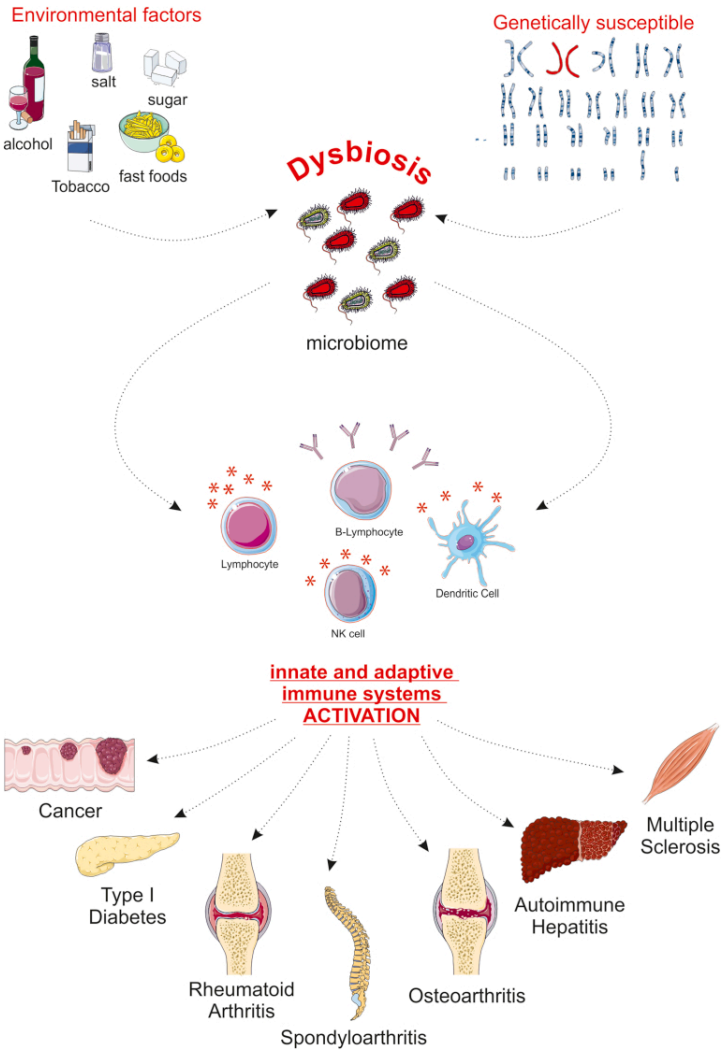
Gut microbiota are the naturally occurring organisms in the human gut once referred to as "gut flora". The human gut microbiota consists of trillions of cells including bacteria, fungi and viruses which exist as a dynamic population and have an influence on homeostasis and disease (Thursby and Juge 2017). The gut microbiome is a term referring to the sum of these microorganisms with their genetic material, and products of their metabolism at any one time.

Carabotti et al (2015) suggest that the gut microbiome can interact with the central nervous system through bidirectional signaling.

It is thought that the diet in infancy establishes the initial microbiota and this is altered throughout life by dietary habits. Divergence from the "normal" gut microbiota composition is referred to as dysbiosis and may occur after antibiotics (Ferreyra et al. 2014) or in the presence of pathology (Lopez-Siles et al. 2017). As well as being the result of pathology, dysbiosis may be the cause of pathology for example the expansion of a pathogenic colony in the gut after antibiotics (Ferreyra et al. 2014) or it is possible that dysbiosis may trigger pathology elsewhere in the body such as cardiovascular

disease, asthma, metabolic syndrome, and obesity (Carding et al. 2015). The interaction between the microbiome and dysbiosis and chronic diseases have been summarized in Figure 10.

Figure 10 The potential interactions involved in dysbiosis (Szychlinska et al. 2019).



Indeed, it is possible that interactions between microbiota and the host are reciprocal. In patients with multiple sclerosis the gut microbiome is significantly different to people without multiple sclerosis (Kirby and Ochoa-Repáraz 2018). For example, in multiple sclerosis patients *Akkermansia muciniphila* and *Acinetobacter calcoaceticus* are increased while *Parabacteroides distasonis* is reduced *(*Cekanaviciute et al. 2017). Also reduced are other bacteria such as Clostridia, Actinobacteria and Bacteroides which are capable of limiting inflammation (Tremlett and Waubant 2017).

Some microbiota appear to favour an environment which is pro-inflammatory, while other microbiota favour an anti- inflammatory environment. Diet appears to affect the balance between these two states. Low fibre Western-style diets for example, seem to lead to a proliferation of certain microorganisms. On the other hand, a quite different colony of microorganisms proliferate when the diet is more vegetable-based, as is the case in the Mediterranean diet. One suggestion is that the high fibre diet provides a better environment for these particular types of microorganisms. Riccio and Rossano (2018) suggest that the microorganisms that flourish in the vegetable-based diet exhibit protective and anti-inflammatory properties. They might do this by reducing oxidative stress and inhibiting nuclear factor kappa B which is a proinflammatory transcription factor. Multiple sclerosis is a disease whose pathophysiology involves inflammatory processes. It is feasible that the gut microbiota of multiple sclerosis patients is a factor in the cause of the disease or a result of the disease. Either way, it is associated with the promotion of inflammatory cytokines and overall stimulation of inflammation. A diet such as the Mediterranean diet might alter the microbiota to produce a microbiota that provides a protective and anti-inflammatory outcome. If there were more evidence to support any potential health benefits, then it might be reasonable to suggest dietary interventions as a prevention measure.

1.2.10 *Multiple sclerosis in Cyprus*

Middleton and Dean (1991) studied prevalence of multiple sclerosis in three areas of Cyprus. The Greek speaking district of Paphos, the eastern Famagusta area and an area in the Troodos mountains. They suggested the prevalence of multiple sclerosis in Cyprus was 44.5 per 100,000. In a study sponsored by the United Nations, Dean et al. (1997) studied the prevalence of multiple sclerosis in the Turkish-speaking occupied area and the Greek-speaking Republic of Cyprus. It was found that the prevalence of multiple sclerosis was highest in Turkish-speaking Cypriot men. The prevalence in the Greek-speaking part of Cyprus, was 42 per 100,000, with males 39 per 100,00 and females 46 per 100,000. Charalambidou Pantzaris and Patrikios (2016) studied patients from CING using data from 2000-2014. They used records of patients attending during this period and estimated the total individual multiple sclerosis patients based on appointments. Their estimate of prevalence of multiple sclerosis in The Republic of Cyprus was 198 per 100,000. It is possible that the 1991estimate of prevalence had under-recorded the true prevalence and/or the 2016 estimate of prevalence is an over estimate, although there is no

evidence to support either suggestion being the case. It is also possible that diagnosis has improved with time due to advances in technology.

Thesis structure

Chapter one is the introduction and includes a description of multiple sclerosis as well as an introduction to the study and Cyprus. The literature is reviewed in Chapter 2 with emphasis on food stuffs, diet and in particular The Mediterranean diet. Chapter 3 describes the aims and objectives of the study while Chapter 4 describes the methodology used. The results are presented in Chapter 5 and discussed in Chapter 6 together with conclusions and ideas for future research. All the references cited are listed in "The Reference Section". Finally, there are Appendices which include the questionnaire in English and Greek as well as all the official forms such as the consent form, participation form, ethics approval, and debrief forms in English and Greek. Tables of results not included in the thesis can also be found in the appendices.

Chapter 2. Literature Review

Insufficient published papers on the risk of multiple sclerosis and the Mediterranean diet prevented a "standard" systematic review using the PRISMA protocol (Moher et al. 2015). Instead, the literature was reviewed in relation to the Mediterranean diet and health and the risk of multiple sclerosis where available.

The review of literature began with a brief history of the Mediterranean diet and included the composition of the diet and the concept of the Mediterranean diet food pyramid. The various foods that are included in or excluded from the diet were reviewed in the historical context. Then the concept of "The effects of Adherence to the Mediterranean diet" were reviewed along with a brief mention of other foods and "Western diets". The suggested health benefits of the Mediterranean diet were examined and the evidence for anti-inflammatory properties of the Mediterranean diet were reviewed. The association of the Mediterranean diet and the risk of multiple sclerosis were then reviewed.

Literature on potential confounders were next reviewed. More than forty environmental factors have been suggested as risks to acquiring multiple sclerosis (Belbasis et al. 2015), and some of these risk factors may be confounders in this case-control study. According to the Boston University School of Public Health (2013) Three conditions must be present for confounding to occur:

1. The confounding factor must be associated with both the risk factor of interest and the outcome.
2. The confounding factor must be distributed unequally among the groups being compared.
3. A confounder cannot be an intermediary step in the pathway from the exposure of interest to the outcome of interest.

The possible confounders are reviewed in detail while unlikely confounders are briefly mentioned. The more likely confounders include sex (gender), obesity, smoking, and socioeconomic status. Finally, other suggested associations unlikely to be confounders in this study are briefly listed. The literature search method is summerised below.

Literature Search Methods

Databases

PubMed and Google Scholar

Original articles of research studies published up until 1st September 2019.

Search terms

"Multiple Sclerosis", Mediterranean diet", "diet", "alcohol", "gender", "female", female gender", "smoking", "passive smoking", "shisha-pipe/narghile/waterpipe /hookah-smoking", "cigarette- smoking", "tobacco smoking ", "pipe-smoking", "cigar-smoking", "obesity", "socioeconomic status" "aetiology", "etiology", "risk",

Boolean strings

"or" and "and".

Inclusion

Only papers written in English were included.
The references of any papers identified were then searched for additional papers that had potentially been missed.

Exclusion

All studies found to involve animals.
Papers on childhood/paediatric multiple sclerosis.
Papers dealing with disease progression, drug therapy and other treatment.
All other papers where a diagnosis of multiple sclerosis had not been formally made by a neurologist

Literature Search Results

Databases	
PubMed	
Multiple Sclerosis	85,281
Multiple sclerosis and gender	5,212
Multiple Sclerosis and diet	802
Multiple Sclerosis and Mediterranean diet	21
Multiple sclerosis and Smoking	615
Multiple sclerosis and Obesity	354
Multiple sclerosis and socioeconomic status	320

Databases	
Google Scholar	
Multiple Sclerosis	902,000
Multiple sclerosis and gender	425,000
Multiple Sclerosis and diet	204,000
Multiple Sclerosis and Mediterranean diet	14,900
Multiple sclerosis and Smoking	150,000
Multiple sclerosis and Obesity	147,000
Multiple sclerosis and socioeconomic status	26,800

As a result of the inclusion and exclusion criteria, papers that examined disease progression and the Mediterranean diet were excluded. The literature search found only one unique study that examined the Mediterranean diet and the aetiology of multiple sclerosis and six studies relating to diet generally and the aetiology of multiple sclerosis. Many other papers were reviews, systematic reviews and meta-analyses.

2.1 The Mediterranean diet

2.1.1 *The Mediterranean diet history*

Ancel Keys might be credited with the recent interest in the Mediterranean diet in the 20th Century, but the diet consumed around the olive growing region of the Mediterranean basin has a history predating Biblical times. Agriculture or the domestication of crops occurred between 10000 to 4000 BCE and was dictated by climate and religious practice (Berry et al. 2011). The term Biblical diet has been coined to refer to the food consumed during the biblical period from the time of the Patriarchs until King David in Jerusalem from 1750 to 1100 BCE (Berry et al. 2011). Both archaeo-botany and written records have provided evidence of foods eaten during this period and their influence on the current Mediterranean diet. The Bible provides written evidence of the so-called "seven species" – wheat, barley, grapes, figs, pomegranates, olives and date honey (Deuteronomy 8:8). These staples can be found in variations of the Mediterranean diet still today. There is also hieroglyphic evidence of wine and olive exports from Canaan to Egypt (both components of the current Mediterranean diet) (Berry et al. 2011).

The first five books of The Jewish Torah and Christian Bible were probably written over a couple of centuries around the 6-7th BCE. These books make many references to food, agriculture and animal husbandry. The so-called Pentateuch diet refers to the diet mentioned in these books and has some similarities to the Mediterranean diet since both are characterised by a diet based mainly on whole-grain cereals, fruits, nuts, vegetables, legumes and a moderate consumption of fish, poultry, dairy products, and a daily glass of red wine taken with food. The Mediterranean and Pentateuch diets both limit the consumption of red meats and sweets, to festivals and feasts (Kastorini et al. 2011). There are large quantities of plant-derived foods consumed. These are mainly, vegetables, legumes, nuts, fruits and seeds, and whole grain cereals. Seafood and some dairy products especially yogurt and cheese are also eaten. However, there is low consumption of sweet desserts and whole milk, butter or cream (Issa et al. 2011). Sweet tastes in foods are derived from figs and carobs and there are virtually no refined sugars. The consumption of poultry and eggs is also limited (Martínez-González et al. 2015). The diet may include one glass of wine daily (especially red wine), which is usually taken with meals (Lamuela-Raventós and Andres-Lacueva 2004; Rodríguez-Morató Boronat Dierssen and de la Torre 2018).

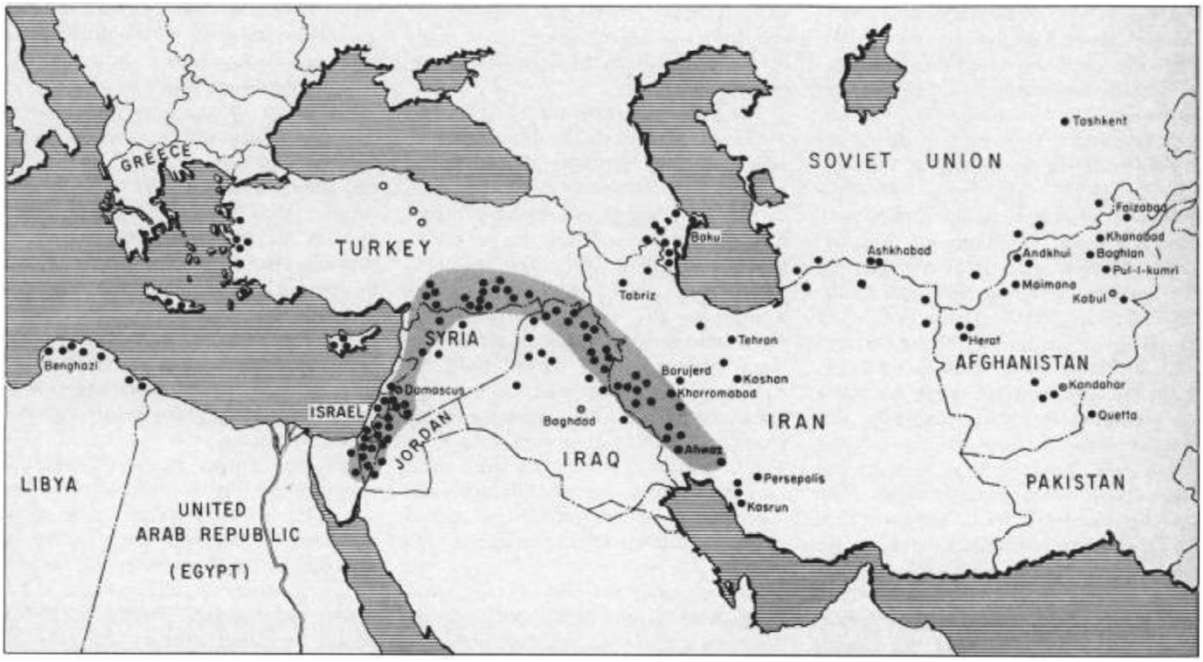
Cultivation of wild olive trees appears to have a history of at least 6,000 years, according to religious texts and archaeological discoveries. For example, archaeological studies by Zohary & Spiegel-Roy (1975) suggest evidence exists of domestication of the wild olive

to *Olea europaea L.* around 4000 BC in Palestine and cultivation gradually continued from East to West. This was most likely carried out by the Phoenicians, Etruscans, Greeks and Romans (van Zeist 1980). The name *Olea europaea L. sativa* has been used to distinguish it from the wild olive subspecies *oleaster* (Lavee, 1996).
Figure 11 shows the probable spread of cultivated olive trees from East to West in the Mediterranean basin (http://www.mikpens.com/mediterraneanolivehistory.html).

Figure 11 The probable spread of cultivated olive trees from East to West in the Mediterranean basin. Source (http://www.mikpens.com/mediterraneanolivehistory.html

The domestication of crops in Western civilization most likely took place from ca. 10,000 to 4000 BCE. (Berry et al. 2011). To place this in context, the invention of the wheel, the development of writing and the use of metals came later around 4000 to 1000 BCE. The evolution of civilization and domestication of crops is thought to have originated in the "Fertile Crescent" which refers to fertile soil bordering the rivers Nile, Tigris and Euphrates and includes parts of modern-day Egypt, Lebanon, Israel, Jordon, Syria, Iran and Iraq which can be seen in Figure *12* (Harlan and Zohary 1966). The progenitor of cultivated wheat is Wild Emmer and the distribution follows the "Fertile Crescent".

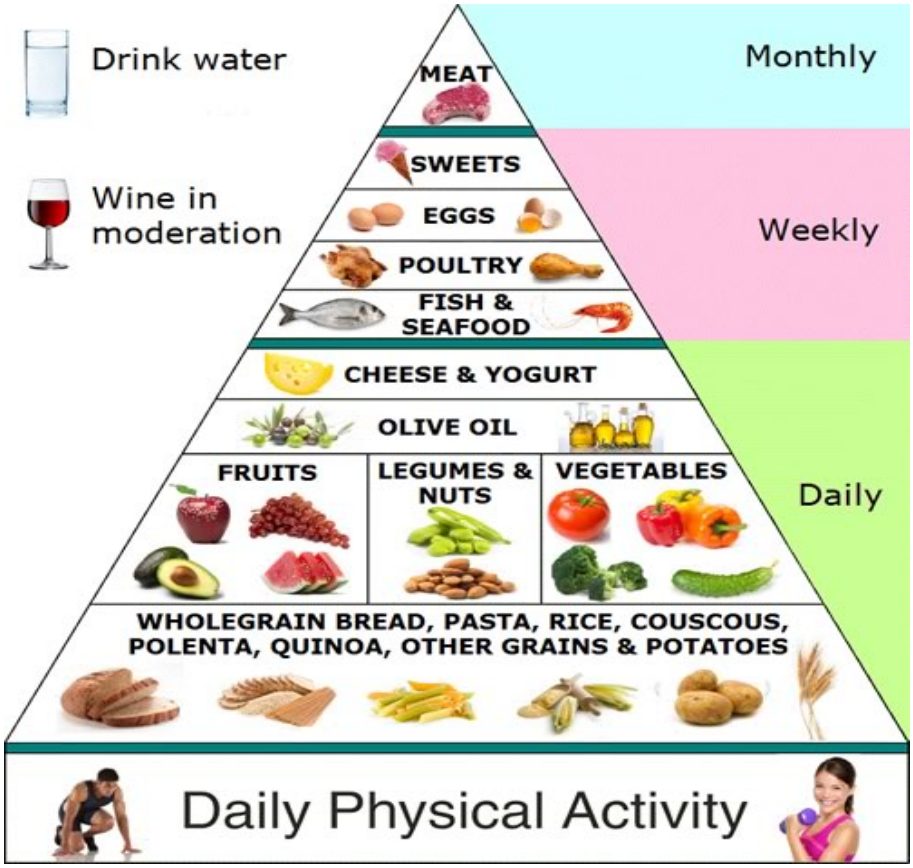
Figure 12 The distribution of wild emmer (Triticum dicoccoides) in the Near East. Solid circles represent known sites in which wild emmer are common (Harlan and Zohary 1966).



2.1.2 *The Food Pyramid*

The Mediterranean diet pyramid is based on food typical of the Greek island of Crete. The three Pyramids used mainly in research are those devised by "The 1999 Greek Dietary Guidelines" (1999), "Oldway's Preservation and Trust" (2009), and "The Mediterranean diet foundation" (2011). As an example, Figure 13 shows a food pyramid from Oldways Preservation & Exchange Trust (2009).

Figure 13 An example of The Mediterranean diet food pyramid (image credit oldwayspt.org © 2009 Oldways Preservation & Exchange Trust)



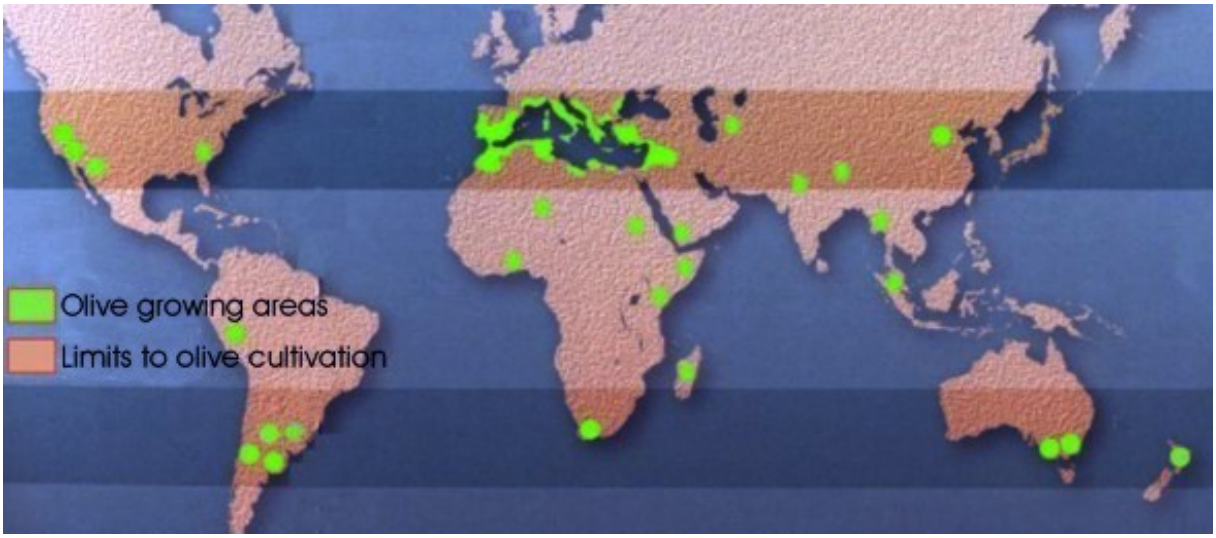
The Mediterranean diet might be considered as a lifestyle choice not just a diet pattern (Bach-Faig et al. 2011). The foods that ought to be eaten in the largest amounts and the lifestyle activities most important in the Mediterranean diet appear at the bottom of the pyramid whilst foods which should be eaten rarely or in moderation are placed at the top (Willett et al. 1995). It is difficult to define the absolute components of the Mediterranean diet as the constituents can be variable for example, geographically, and the diet can still fall into the category of the Mediterranean diet. One current definition is that the Mediterranean diet is based on consumption of extra-virgin olive oil, unrefined cereals, legumes and diverse vegetables (in particular tomatoes) (Riccio and Rossano 2015). Olive oil is used for cooking and in salads and is the main source of fat. The Mediterranean diet "lifestyle choice"(Bach-Faig et al. 2011) has at its foundation daily physical activity. The major food components are plant based, with daily consumption of fruits, vegetables, legumes, nuts and whole grains. Extra virgin olive oil is the

predominant fat used in cooking and food dressings. Dairy products, poultry and eggs are eaten in moderation while sweets and red meat are eaten monthly or perhaps limited to festivals.

Olives

Olive growing requires a Mediterranean climate, which typically features warm, wet winters and hot, dry summers. These conditions can be found in the Mediterranean basin and parts of South West Australia and also California, Chile and South Africa. As a result, areas with this type of climate account for the geographical distribution of current olive cultivation as shown in Figure 14.

Figure 14 The Geographical distribution of current olive cultivation Source (http://www.internationaloliveoil.org/projects/paginas/Section-a.htm).



Olives can be eaten as fruit immediately after curing, once the bitter compound Oleuropein has reduced (Johnson et al. 2018), or they may be canned or bottled and eaten later. Alternatively, they may be used to produce olive oil. Ripe olives are picked and taken to an olive mill where they are washed and crushed. The resulting paste releases oil droplets in a process known as maceration (Olive Times 2018). Extra virgin olive oil is the top grade of olive oil and is produced by centrifuge spinning that pulls out the oil and water. Whereas with lower grade refined oil, the extraction process uses heat or chemicals. Removing the water then leaves the oil (California Olive Ranch 2018). Olives and their products are important components of the Mediterranean diet with supposed health benefits which are discussed later. Extra virgin olive oil is considered the most important of the various olive oils and is dealt with in detail later.

Grains

The Gramineae family of grasses (which includes barley, oats, maize, rice and wheat) have been grown in the Mediterranean area for thousands of years. The seeds of these plants are harvested to make cereal (Seal et al. 2006). Wheat and barley nn the Pentateuch diet, and are major components of The Mediterranean diet which also includes rye and oats. Barley was traditionally harvested in the spring and wheat in the summer.

A whole grain consists of three parts: 1. The outer bran or aleurone layer. 2 The endosperm and 3. The germ (Ross et al. 2015). Grains cannot usually be digested raw, so they must be flaked, cracked, puffed, popped or ground before being consumed. They also include inedible parts such as the hull and husk which must be removed during processing to make the grains edible (Seal et al. 2016). Grains are either intact or processed. Even if processed, to be called whole grain, it must contain all three components. So, intact whole grains are grains in their natural state, with all three parts of the grain still intact and in their original proportions. Grains are rarely consumed in this form since they are difficult to chew and digest. While a processed version must contain all three parts of the grain, it will have been milled into flour instead of being left intact. Some examples of whole grain foods include 100% whole wheat bread, wholemeal pasta or crackers. The amount (%) and ratio of bran endosperm and germ varies in the processed version compared with the intact whole grain. This has led to food labelling issues with manufacturers and processors (Van Der Kamp et al. 2014). The consortium of the HEALTHGRAIN EU project identified the need for developing a definition of whole grain to avoid this variability. Indeed, they proposed a definition of whole grain as an ingredient as follows: “Whole grains shall consist of the intact, ground, cracked or flaked kernel after the removal of inedible parts such as the hull and husk” (Van Der Kamp et al. 2014). Refined grains are the most highly processed form of grains and examples of foods made from them include white bread, white pasta, white rice, and pearled barley. Although these refined grains start out as whole grain, the milling processes removes the bran and germ to ensure a finer textured whiter flour (in the case of wheat) with longer shelf life but this results in lower dietary fibre, iron, and many B vitamins (Ferruzzi et al. 2014). Some producers of refined grain add back certain vitamins and minerals under fortification regulations, but this is variable between countries. Enriched grain means nutrients that were lost during grain processing have been added back such as flours enriched with germ. Fortified means vitamins or minerals have been added to a food that were not originally in the food.

Fruits and nuts

Fruits are an integral part of the Mediterranean diet and may include citrus fruits, such as oranges, lemons, grapefruit in addition to apples and pears. Figs grow on the ficus tree (*Ficus carica)* and were one of the first fruits to be cultivated in the Mediterranean region and remain an important fruit in the Mediterranean diet. The persimmon (Diospyros kaki, Ebenaceae) originated in China and has been cultivated in the Mediterranean basin for over a hundred years (Tous and Ferguson 1996). Other fruits common to the traditional Mediterranean diet include apples, apricots, avocados, cherries, clementines, grapefruits, grapes, melons, nectarines, peaches, pears, strawberries, tangerines and tomatoes (Davis et al. 2015). Tree nuts are dry fruits with one seed. At maturity, it is the ovary wall that becomes hard. Nuts are integral components of the Mediterranean diet, especially almonds, hazelnuts, pine nuts, pistachios, and walnuts (Ross et al. 2015).

Vegetables

Traditionally, the vegetables eaten in the Mediterranean diet depend on those that flourish in the soils and climate of that region and are often grown for raw consumption in salads. For example, Greek salad may include tomatoes, cucumber, peppers, olives (which are all technically fruits), onions along with pickled capers which are the flower buds of *Capparis spinose*. The fresh salad is usually dressed with olive oil. Other vegetables common to the traditional Mediterranean diet include brussels sprouts, cabbage, peas, leeks and turnip. These vegetables are more likely to be cooked than eaten raw.

Fish

Fish and shellfish have been caught around the Mediterranean coast for thousands of years and fish consumption is regarded a major component of the Mediterranean diet (Willett et al. 1995). The fish species found in the Mediterranean region include: anchovy, common sole, gilthead sea bream, hake, mackerel, sardine and sea bass. Of these, anchovies, sardines and mackerel are oily fish. These fish are a good source of vitamin D.

Legumes

There are over 18,000 species of legumes (Sprent and Platzmann 2001). They are key components of traditional diets (Messina 1999) such as the Mediterranean diet and include lentils, chickpeas, beans, peas, flat beans, split peas and peanuts. Essid (2012) states that the Romans (between the 7-3rd centuries BCE) spread the cultivation of legumes and agriculture generally at this time was helped by the Roman introduction of irrigation systems. Legumes which are a good source of protein were eaten in areas where cattle could not be reared, or meat was too expensive.

The health benefits of legumes are discussed in a later section, but it is worth noting that Darmadi-Blackberry et al. (2004) suggest a higher intake of legumes are a dietary predictor of survival among elderly people when compared with other food groups.

Alcohol, especially red wine

Red wine has been produced in the region for centuries and small quantities (1-2 glasses) are often drunk with meals. Alcohol and red wine are discussed in detail later.

Low consumption of red meat and processed meat

The consumption of red meat and processed meat in the Mediterranean diet is typically small and is usually restricted to a couple of times per month or at festivals.

Low consumption of poultry, and dairy products

Small amounts of poultry and dairy produce in the Mediterranean diet reflect the historical scarcity of these foods in the area.

2.1.3 *Epidemiology*

Since the early 1960s, there has been extensive epidemiological research on the individual elements of the Mediterranean diet studying disease risk or disease progression and reduced mortality. The hope was that any food or item of food that was found to be beneficial could be added to the Western diet to improve health outcomes. However, studies have failed to find statistically significant associations with separate components of the Mediterranean diet (Hernández Ruiz et al. 2015). More recent research has focused on the effect of adherence to the Mediterranean diet as a whole (Martínez-González et al. 2015). The term "adherence" has been coined to denote how closely a person follows the Mediterranean diet, usually described using a diet scoring system. A high Mediterranean diet score suggests the person is following the Mediterranean diet more closely than a person with a low Mediterranean diet score. It has been suggested that the elements of the Mediterranean diet act synergistically, and it is the whole rather than the parts that are important (Trichopoulos and Trichopoulo 2008).

Various indices have been published to assess the effect of adherence to the Mediterranean diet. The first index was first proposed in 1995 by Trichopoulou et al., who were assessing the adherence to the Mediterranean diet and mortality risk in an elderly Greek population. There were eight components to this index; a ratio of quality of fat and seven food groups present in the diet. The seven food groups included: vegetables, legumes, grains, fruits and nuts, meat and meat products, milk and dairy products and red wine intake. Later, this index was modified to include fish intake (Trichopoulou et al.

2003). The effect of adherence to the Mediterranean diet can be assessed using the 9-Unit dietary score proposed by Trichopoulou (Trichopoulou et al. 2003) while other indices use slight modifications.

An example of a 9 component Mediterranean diet adherence score such as that used by Trichopoulou uses the following foods:

Fruit and nuts
Vegetables
Legumes
Monounsaturated fats from olive oil
Fish
Red wine
Grains
Low consumption of red meat and processed meat
Low consumption of poultry, and dairy products

On the other hand, some indices do not share important aspects of its definition due in part to different populations, and the age of people studied. This gives rise to a large number of different scoring systems in existence (Hernández Ruiz et al. 2015) and makes comparison of different studies sometimes difficult.

For example, some scores include lifestyle factors, while others do not.

Hernández Ruiz et al. (2015) reviewed the literature for adherence to the Mediterranean diet and examined evidence for lower mortality risk when the Mediterranean diet was adhered to. They found that while a whole Mediterranean diet showed significant associations with reduced mortality in several studies, there were no statistically significant associations with separate components of the same diet to reduced mortality. Several studies have used Trichopoulou's Mediterranean diet index (or modifications of it) (Bamia et al. 2017).

In order to calculate an individual's Mediterranean diet score the individual provides a record of their diet, usually over several days or through the use of a diet recording tool such as a Food Frequency Questionnaire (FFQ). Each of nine components of the Mediterranean diet are assigned a value of 0 or 1 with the use of the sex-specific median intake of the food as the cut off. A value of 1 is assigned to the diet where consumption of the 5 expected beneficial food components (vegetable, legumes, fruits/nuts, cereals and fish) is equal or above the sex specific median intake level and a value of 0 is assigned when the consumption is below the median intake level. For meat, poultry, and dairy products (which are presumed detrimental) a value of 1 is assigned to the diet of participants where consumption is below the sex specific median intake level and 0 when

it is above the median intake level. A value of 1 was assigned to the diet of male participants who consumed between 10 and 50g of alcohol per day and to the diet of women who consumed between 5 and 25g of alcohol per day. Therefore, using this 10point index, a total Mediterranean diet score would range from 0 (minimal adherence to the traditional Mediterranean diet) to 9 (maximal adherence). It is then possible to produce categories of 0-3, 4-6 and 7-9 to represent poor adherence, average adherence and high adherence to the Mediterranean diet (Mirmiran et al. 2015). The sex-specific median intake level is calculated for each of the 9 components, using data from the whole group ie cases and controls. For example, the weekly amount of (125ml) glasses of red wine is recorded for males and females in the study population and the median value for each gender calculated. The diet of people where alcohol intake is above the median value are allocated a score of 1 and those below, a score of 0. This is repeated for each of the other 8 categories of the Mediterranean diet adherence index, giving a total score out of 9.

The Mediterranean diet score devised by Panagiotakos et al. (2007) uses 9 food groups plus Olive-oil in cooking and alcohol. These are represented in Table 2.

Table 2 The Mediterranean diet score devised by Panagiotakos et al. (2007)

Frequency (servings per week) and allocated score						
1.Non-refined cereals	Never =0	1-6 =1	7-12 =2	13-18 =3	19-31 =4	>32 =5
2.Potatoes	Never =0	1-4=1	5-8=2	9-12=3	13-18=4	>18=5
3.Fruits	Never =0	1-4=1	5-8=2	9-15=3	16-21=4	>22=5
4.Vegetables	Never =0	1-6=1	7-12=2	13-20=3	21-32=4	>33=5
5.Legumes	Never =0	<1=1	1-2=2	3-4=3	5-6=4	>6=5
6.Fish	Never=0	<1=1	1-2=2	3-4=3	5-6=4	<6=5
7.Red Meat and products	<1=5	2-3=4	4-5=3	6-7=2	8-10=1	>10=0
8.Poultry	<3=5	4-5=4	5-6=3	7-8=2	9-10=1	>10=0
9.Full fat dairy products	<10=5	11-15=4	16-20=3	21-28=2	29-30=1	>30=0
10.Olive-oil in cooking (times/week)	Never=0	Rare=1	<1=2	1-3=3	3-5=4	Daily=5
11.Alcohol 10 units=1000ml.	<300=5	300=4	400=3	500=2	600=1	>700=0

Intake of each food is estimated, and a score allocated from 0-5 (or the reverse) allocated to each group according to the position of the food group in the Mediterranean diet pyramid. The total Mediterranean diet score is the addition of each group and ranges from 0-55. People with the highest scores are considered to adhere more closely to the Mediterranean diet. For example, a food such as fruit, that is considered to be typical of the Mediterranean diet, is given a score of zero if fruit is never eaten in a week and 5 if it is eaten more than 32 times in a week.

The converse for a food such as red meat, that is not considered to be typical of the Mediterranean diet, a score of 5 is given if the red meat is never eaten in a week and 0 if it is eaten more than 10 times in a week.

2.2 The Mediterranean diet and health benefits

Health benefits of the Mediterranean diet were first proposed by the American doctor Ancel Keys in 1960 (Keys 1995). He noticed that coronary heart disease incidence was lower in certain parts of Southern Europe where people were eating a mainly vegetable-based diet. Since this work, there have been a number of studies that primarily focused on the single nutrients or single parts of the Mediterranean diet in relation to the occurrence of disease. The hope was to find the beneficial elements of the Mediterranean diet and to add these to a non- Mediterranean diet to improve health (Keys 1995). Ancel Keys is well known for his "Seven Countries Study" which was an epidemiological longitudinal study that examined the relationships between lifestyle, diet, coronary heart disease and stroke in seven countries (USA, Finland, Greece, Japan, The Netherlands, Italy and the former Yugoslavia) (Menotti et al. 1989). The study found an increased risk of heart disease in subjects following a "Western diet pattern" compared with subjects following a Mediterranean diet. Sofi et al. (2008) systematically reviewed all the prospective cohort studies investigating the association between adherence to a Mediterranean diet and health outcomes up to June 2008.

The meta-analysis concluded that adhering to the Mediterranean diet reduced age-adjusted mortality from cardiovascular diseases by 9% and cancer by 6% while the incidence of Parkinson's disease and Alzheimer's disease was reduced by 13%. A review by Dinu et al. (2017) concluded there was "robust evidence for overall mortality, cardiovascular diseases, overall cancer incidence, neurodegenerative diseases and diabetes". The Mediterranean diet also appears to reduce the risk of other diseases caused by chronic inflammation (Gotsis et al. 2015).

2.2.1 *Evidence for the anti-inflammatory action of the Mediterranean diet*

The Mediterranean diet is considered to be anti-inflammatory (Martínez-González et al. 2015) and a considerable amount of evidence supports this. For example, Chrysohoou et al. (2004) randomly recruited 1,514 men and 1,528 women between 2001 to 2002. They found that adherence to the traditional Mediterranean diet was associated with a reduction in the concentrations of blood inflammation and coagulation markers. A meta-analysis also suggested that a Mediterranean diet decreases inflammation (Schwingshackl and Hoffmann 2014).

Multiple sclerosis is a disease characterised by chronic inflammation (Pantazou Schluep and Du Pasquier 2015) and the Mediterranean diet is considered to be anti-inflammatory. It is possible that those eating an anti-inflammatory diet could be protected from a chronic inflammatory disease like multiple sclerosis while those not eating a Mediterranean diet could be at a greater risk of acquiring multiple sclerosis. However, there is a paucity of case-control studies examining the effect of Mediterranean diet adherence with risk of acquiring multiple sclerosis. The literature search found only one such study, which was an Iranian hospital-based study. The study included 70 patients with multiple sclerosis and 142 hospital-based controls. The multiple sclerosis cases were compared with controls in relation to adherence of the Mediterranean diet, using a 9-unit dietary score similar to that of the Trichopoulou Mediterranean diet index. The results showed that higher consumption of fruits reduced the risk of multiple sclerosis (OR=0.28, 95% CI: 0.12-0.63, *p*-value: 0.002). While higher vegetables intake also reduced the risk of multiple sclerosis (OR=0.23, 95% CI: 0.10-0.53, *p*-value: 0.001) They concluded that the Mediterranean dietary pattern is associated with a reduced risk of multiple sclerosis (Sedaghat et al. 2016). The study included many more female than male multiple sclerosis cases (57 female to 12 male), giving a female to male ratio of 4.7:1 which is much higher than the world ratio in 2000 of 2.3:1 (Alonso and Hernán 2008). The controls (114 female to 26 male). Controls were frequency-matched with cases by sex and age (5-yr age groups). There is no indication if the sex ratio of cases is representative of the gender ratio of multiple sclerosis in Iran or whether the reduced risk of multiple sclerosis in those people adhering to a Mediterranean diet would be equally as great in a male population since such a small number of cases (12) were male. Since people in Iran are not expected to consume alcohol, this element of the Mediterranean diet score was

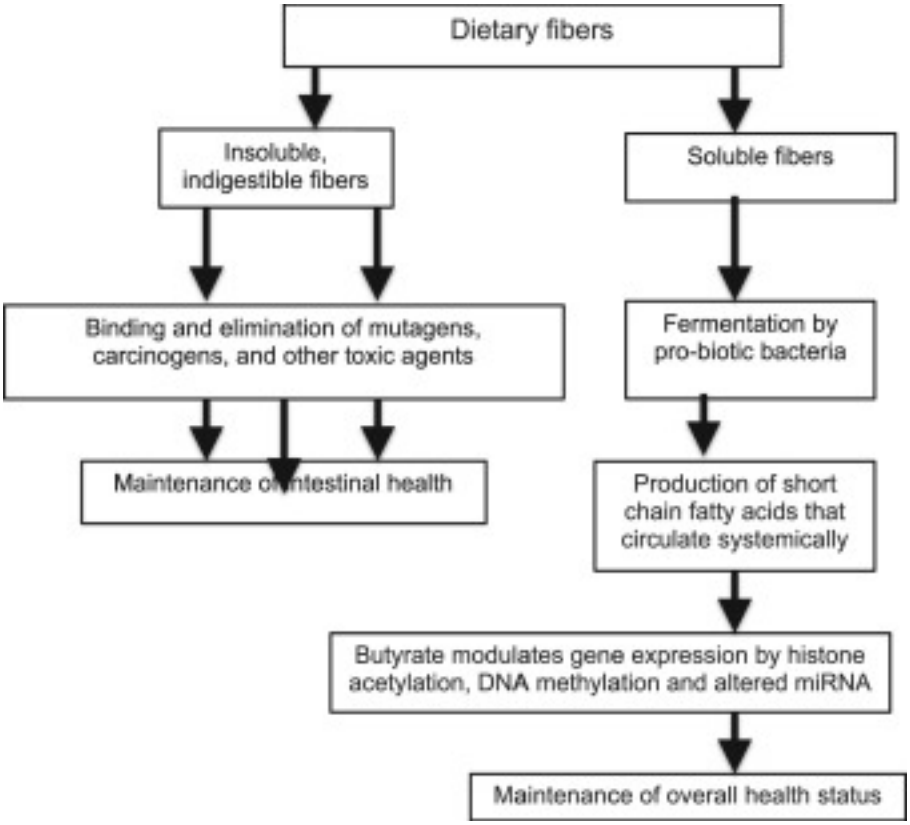
omitted. This makes any comparison with this study more difficult as the elements of the score are different.

Black et al. (2018) conducted a case-control study and identified two major dietary patterns – healthy and Western. Whilst not The Mediterranean diet, the healthy diet was high in vegetables, legumes, poultry, fish, eggs, while the Western diet was high in meat, full-fat dairy and low in whole grains, nuts, fresh fruit, and low-fat dairy. An FFQ was completed by 252 multiple sclerosis cases and 446 controls. After various adjustments (history of infectious mononucleosis, body mass index, serum 25-hydroxyvitamin D concentrations, smoking, education and race), they found that a one-standard deviation increase in the healthy pattern score was associated with a 25% reduced risk of a first clinical diagnosis of central nervous system demyelination (adjusted odds ratio 0.75; 95% confidence interval 0.60, 0.94; p=0.011).

Fibre and short chain fatty acids

Prasad and Bondy (2019) in their review suggest that there is much observational evidence that high-fibre diet reduces the risk of developing cardiovascular disease, (Lattimer and Haub 2010) and colon cancer (Wong et al. 2006). While Zhao et al. (2018) suggest gut bacteria can be selectively promoted by dietary fibre and this can alleviate type 2 diabetes.
Dietary fibre can be categorised as either insoluble, indigestible fiber or soluble as depicted in the flow diagram below.

Flow diagram to categorise dietary fibre (Prasad and Bondy 2019)



Cereals contain more insoluble than soluble fibers, whereas fresh fruits and vegetables contain more soluble fibre (Ötles and Ozgoz 2014). Both soluble and insoluble fiber have their own benefits. Insoluble fibre includes cellulose, some hemicellulose, and lignin which may bind and eliminate toxin, carcinogens and mutagens (Soliman 2019). Soluble fibre is found in oat bran, barley, nuts, seeds, beans, lentils, peas, and some fruits and vegetables. It attaches to cholesterol particles reducing the overall cholesterol level and lowers the risk of heart disease. After fermentation in the gut, small chain fatty acids are produced which have the ability to modulate gene expression.

In addition to their individual beneficial properties there appears to be interaction between insoluble and soluble fibres in the prevention of Cardiovascular Disease (Mudgil 2017).

Later in the chapter, the biome is discussed and the effect fibre plays in disease and health.

2.2.2 *Possible mechanisms*

Vegetables, fruit and nuts

While a synergistic action has been suggested to explain the benefits of the Mediterranean diet, individual foods within the diet have been recognised as having health benefits. Pekmezovic et al. (2009) suggested a protective role of fruits in the aetiology of multiple sclerosis, while Bagheri et al. (2014) suggested a protective role of fruits and low-fat dairy products in the aetiology of multiple sclerosis. For this reason, individual foods and food groups were examined.

The reason why these foods have been commented on, is that they are foods commonly consumed in Cyprus, and that they could be considered part of a Mediterranean diet, and that they potentially have effects on the inflammatory response which may be relevant in MS.

The high carotenoid and flavonoids content of fruits have been shown to be inversely associated with intima-media thickness which is a prognostic marker for subclinical atherosclerosis (Mursu et al. 2007). Ciccone et al. (2013) reviewed the literature and found many data supporting the anti-inflammatory action of carotenoids and their protective effect on cardiovascular events. There is a paucity of research examining dietary carotenoids and multiple sclerosis. Miller et al. (2019) reviewed the literature and concluded flavonoids can play an important role in the management of multiple sclerosis, possibly by reducing the expression of pro-inflammatory cytokines such as IL-1beta and IL-6 and TNF alpha.

Phenolic compounds have antioxidative roles, anticarcinogenic, anti-inflammatory and antimicrobial activities and may have antimutagenic properties. Polyphenol concentrations are some of the highest found in commonly consumed fruits and figs are also an excellent source of dietary fibre as well as minerals, vitamins and they are also fat and cholesterol-free (Solomon et al. 2006). It is suggested that the skin of a fig is a major source of anthocyanins and polyphenols and there are benefits of not discarding fig skins before consuming the fig (Solomon et al. 2006). There are numerous papers which have shown that figs, which are commonly eaten in Cyprus, may have a beneficial impact on inflammatory responses. Some of this evidence has recently been reviewed by Arvaniti et al. (2019) who presented the phytochemical compounds found in both fresh and dried fig varieties and discussed the potential antioxidant effects which may have a beneficial effect on health. The authors also reported contradictory results in the literature regarding the effect of air- and sun- drying on the total content of phytochemical compounds, as

well as on the concentrations of individual phenolic compounds and carotenoids. The review concluded the antioxidant capacity of figs was highly correlated with the amounts of phenolic compounds.

Dates are the fruits of the date palm *Phoenix dactylifera* and may be eaten fresh or dried and stored for later consumption or in various processed forms (Chao and Krueger 2007).

Citrus fruits include oranges, mandarins, lemons grapefruit and limes. The mandarin (Cit*rus reticulata, Rutaceae*) is the largest of the edible citrus fruits and was introduced to Europe and North Africa in the 16th Century (Tous and Ferguson 1996). Pro-inflammatory enzymes, such as induced Nitric Oxide synthase (iNOS) and cyclooxygenase (Cox-2), have been shown to be responsible for the elevated levels of NO and prostaglandins (PGs), in the pathogenesis of many chronic diseases including multiple sclerosis, Parkinson's and Alzheimeŕs disease and colon cancer due to the inflammatory cytokines tumour necrosis factor-alpha (TNF-α), interleukin-1β and interleukin-6 as well as those mentioned above (Heiss et al. 2001). Similarly, pro-inflammatory cytokines such as IL-1beta and IL-6 and TNF alpha are found in multiple sclerosis so flavonoids, coumarin and volatile oil from citrus fruit exhibit anti-inflammatory activity, may protect against or ameliorate this chronic inflammatory process (Lv et al. 2015).

Beneficial effects of olives and olive oil consumption on health were reviewed by Guo et al. (2018). They concluded that phenolic components and other antioxidants in olive products including oil, were responsible for some of the benefits. Fernandes et al. (2020) undertook a systematic literature review to evaluate the effect of regular olive oil consumption in inflammation which may be relevant in the context of multiple sclerosis. They summarized evidence from randomized controlled trials on the effect of regular dietary intake of olive oil on three inflammatory markers: C-reactive protein, interleukin-6, and tumor necrosis factor-α. They concluded that olive oil consumption reduced the levels of a range of inflammation markers.

A systematic review and meta-analysis by Morvaridzadeh et al. (2020) assessed the effect of pomegranate consumption on oxidative stress parameters. They concluded, based on the systematic review, that pomegranate has positive effects on oxidative stress parameters, but based on the meta-analysis, because of insufficient clinical trials and variable inconsistencies no conclusion could be made on the effect of pomegranate on these parameters.

In their review of citrus fruits, Lv et al. (2015) summerised the anti-oxidative, anti-inflammatory and neuroprotective effects.

Nuts are generally rich in protein, fibre, and polyphenolic antioxidants, and they are high in monounsaturated fatty acids and polyunsaturated fatty acids (Jahromi et al. 2012). They are also excellent sources of copper, iron, zinc, selenium, riboflavin, magnesium, niacin, and folic acid and a diet high in nuts shows an inverse relationship with risk for cardiovascular diseases (Sabaté 1999). Omega 3 fatty acid can also inhibit tumour necrosis factor alpha (TNF-α), and interferon-gamma (INF-γ) and thus potentially suppress demyelination of central nervous system nerve cells which occurs in diseases such as multiple sclerosis (Jahromi et al. 2012). Almonds are part of the prunus family, which are rich sources of mono- and polyunsaturated fatty acids. Sabate and Ang (2009) suggest consuming nuts can reduce the intake of saturated fatty acids (SFA) and increase the intake of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) with a resulting benefit on heart health (Sabate and Ang 2009). Indeed, the US Food and Drug Administration (FDA) has allowed pistachio producers to state the following: “Scientific evidence suggests, but does not prove, that eating 1.5 ounces per day of most nuts, such as pistachios, as part of a diet low in SFA and cholesterol may reduce the risk of heart disease” (US Food and Drug Administration 2003). In addition, pistachios contain high levels of protein, and dietary fibre (Dreher 2012). A review by

Dreher (2012) suggested that potential health benefits of pistachio nuts were because consumption of these nuts may help reduce oxidative and inflammatory stress. While a review by Gorji Moeini and Memariani (2018), suggested the anti-inflammatory properties of almond, hazelnut and walnut might affect pathways in the pathology of neurodegenerative diseases.

Carotenoids are phytonutrients that give the distinctive yellow, orange and red colour to fruits and vegetables. There are at least 700 carotenoids, and some are important food micronutrients (Maiani et al. 2009). Lycopene for example, is found in tomatoes and red peppers contain zeaxanthine, while cucumber contains lutein. The antioxidant properties of these phytonutrients have been suggested as protective against diseases of chronic inflammatory origin (Zakynthinos and Varzakas 2016).

Vegetables

There is strong evidence that eating vegetables is beneficial to health. Meta-analysis indicate that consumption of fruit and vegetables is associated with a reduced risk of cognitive impairment and dementia (Jiang et al. 2017). While a systematic review by Aune et al. (2017) concluded that fruit and vegetables reduced the risk of cardiovascular disease, total cancer and all-cause mortality (Aune et al. 2017).

Whole grains

Whole grains provide carbohydrate, protein, nutrients, vitamins and minerals and can be found in breads such as Rye bread, multigrain bread, wholegrain bread and high-fibre bread made from whole-grain flours (Black et al. 2018). They can also be found in breakfast cereal such as Shredded Wheat, Bran Flakes, Weetabix and porridge and Muesli (Quatela et al. 2018). In population studies, increased intake of whole grain has been shown to be associated with lower risk of cardiovascular disease and type 2 diabetes. Mann et al. (2017) reiterate the benefits of whole grains and suggest a reduction in C-reactive protein (a marker of inflammation) with increased whole grains intake. The American Heart Association guidelines recommend a total dietary fibre intake of 25-30 g per day. They state cardiovascular disease morbidity and mortality can be reduced with a diet high in fibre, such as whole grains (oats and barley) as these produce lipid-lowering effects (Van Horn 1997).

Fish

Finfish and shellfish contain omega-3 fatty acids. It is said that omega-3 fatty acids have anti-inflammatory properties and result in increased cell membrane fluidity. Evidence suggests that these fatty acids have associated cardioprotective, and neuroprotective

properties and decreased risk of some chronic disorders including multiple sclerosis (Hoare et al. 2016; Langer-Gould et al 2018). Hoare et al. (2016), questioned 267 multiple sclerosis cases and 517 controls. They found that there was a reduced risk of a first clinical diagnosis of CNS demyelination in those who had a higher intake (per g/day) of omega-3 polyunsaturated fatty acids (PUFA). This risk reduction was as much as 46% when the omega-3 PUFA was from fish (AOR=0.54 (95% CI 0.31-0.93). Baarnhielm Olsson and Alfredsson (2014) found decreased multiple sclerosis among people eating fatty fish. Fish are also good sources of high-value proteins and have a low fat content. They contain various vitamins (D, A, and B) and minerals such as calcium, iodine, zinc, iron, and selenium (Torpy Lynm and Glass 2006). Some fish may have contamination or have an accumulation of heavy metals such as mercury (Torpy Lynm and Glass 2006). Shark, swordfish, king mackerel, and golden bass are known to be potentially dangerous to pregnant women because of the risk of mercury on the developing foetus (Torpy Lynm and Glass 2006). Dioxins and polychlorinated biphenyls (PCBs) can also accumulate in fish, but the health risks of these chemical levels are considered very low (Torpy Lynm and Glass 2006).

Legumes

Legumes are rich in plant proteins and are considered to be a good source of fibre and carbohydrates. Some may also contain vitamins, calcium, iron, potassium, sodium and phosphorous. Chickpeas for example, have been shown to reduce LDL-cholesterol (Pittaway Robertson and Ball 2008). Carob is a legume which is native to the Mediterranean area and is rich in calcium, potassium and small amounts of iron and vitamin B (Berry et al. 2011). Dietary fibre has been shown to reduce the of risks of chronic diseases such as cardiovascular disease possibly through a mechanism that involves lowering LDL-cholesterol via inhibiting the activity of the enzyme hydroxy-3-methylglutaryl-CoA reductase (Trinidad et al. 2010). Tumour formation in the colon may be prevented by butyrate which is produced by the fermentation of dietary fibre (Fung et al. 2012). This may explain why the Mediterranean diet has been associated with reducing the risk of colon cancer. In a systematic review and meta-analysis Schwingshackl et al. (2017) found that colorectal cancer risk was reduced with increased Mediterranean diet adherence (RR: 0.82, 95% CI 0.75 to 0.88, I^2 = 73%; n = 11 studies).

Alcohol

Alcohol consumption has been suggested as both a risk factor (Pekmezovic et al. 2006) and as a protective factor for multiple sclerosis (D'hooghe et al.2008; Hedström Hillert Olsson and Alfredsson 2014). The suggestion is that red wine is protective in those who smoke, because it attenuates the effect of smoking by reducing acute endothelial damage (Schwarz et al. 2017). Other studies have found no association (Massa O'Reilly Munger and Ascherio 2013). One study found drinking spirits daily was a risk for multiple sclerosis development. The case-control study by Pekmezovic et al. (2006) compared 210 multiple sclerosis cases (Poser's criteria) with 210 age and sex-matched hospital controls. The results showed an OR of 6.7, ($p = 0.026$) suggesting a significant association between consumption of hard liquor per day (daily spirits with no quantities stated) and risk of multiple sclerosis. Hedström et al. (2014) found that alcohol consumption was protective against multiple sclerosis. They used data from two independent population-based case-control studies. The first study was the Epidemiological Investigation of Multiple Sclerosis (EIMS) study. This consisted of a population aged 16 to 70 years in Sweden. Each case was matched by age (predetermined 5-year age groups), sex, and residential area with 2 controls. The study lasted from April 2005 to June 2011. The second study was called the Genes and Environment in Multiple Sclerosis (GEMS) study. Cases fulfilling the McDonald criteria were identified from the Swedish national multiple sclerosis registry. For each case, one control was randomly selected from the national population register and matched by age, sex, and residential area. This study took place between November 2009 and November 2011. The multiple sclerosis cases and controls were different in each study. Participants were categorised as drinkers and non-drinkers. Drinkers were further divided into subgroups of low consumption (<50 g/wk for women and <100 g/wk for men), moderate consumption (50-112 g/wk for women and 100-168 g/wk for men), and high consumption (>112 g/wk for women and >168 g/wk for men). There were no data for former drinkers stated. The alcohol consumption and risk for multiple sclerosis based on EIMS included 745 cases and 1761 controls. While the GEMS study included 5874 cases and 5246 controls. A dose-dependent inverse association between alcohol consumption and multiple sclerosis risk was found in both studies. This was statistically significant in both men and women. In EIMS, women who reported high alcohol consumption had an OR of 0.6 (95% CI, 0.4-1.0) compared with nondrinking women, whereas men with high alcohol consumption had an OR of 0.5 (95% CI, 0.2-1.0) compared with nondrinking men. The OR for the corresponding comparison in GEMS was 0.7 (95% CI, 0.6-0.9) for women and 0.7 (95% CI, 0.5-0.9) for men. The

authors concluded that alcohol consumption exhibits a dose-dependent inverse association with multiple sclerosis which was more pronounced in smokers (Hedström Hillert Olsson and Alfredsson 2014). A meta-analysis of 10 observational studies including 9 case-control studies by Zhu et al. (2015) found no evidence that alcohol consumption was associated with an increased risk of multiple sclerosis. The odds ratios of the association between alcohol consumption and multiple sclerosis was 0.92 (95 % confidence interval 0.73-1.17) overall. A comprehensive review by Wang et al. (2015), concluded that as yet, there was insufficient evidence to draw a conclusion regarding alcohol consumption and multiple sclerosis. With these results, reverse causation needs to be considered. In other words, rather than alcohol consumption (the exposure) leading to multiple sclerosis (the outcome), it is the multiple sclerosis that leads to the alcohol consumption? Voci (2015) highlights the association of alcohol misuse and multiple sclerosis and suggests reverse causation may be due to the psychological impact of the disease.

Wine

Wine and red wine in particular, has been traditionally taken with food in the Mediterranean area for centuries (Arnoni and Berry 2015.). The health benefits of wine have been documented for over 800 years and red wine has been stated as a beneficial component of the Mediterranean diet when consumed daily and in moderation. Polyphenols in wine appear to have antioxidant properties, and red wine has more polyphenols than white. This is due to the fact that in red wine production the grape juice and skin are in contact for up to a month (leading to a partial pickling effect) whereas the contact time is shorter in white wine production (Howard et al. 2002). Red wine contains high levels of flavonols such as anthocyanins, quercetin, catechin, and resveratrol. Resveratrol is found in grape seeds and skins and is said to be protective against *LDL* (Frojdo Durand and Pirola 2008). Similarly, Chiva-Blanch et al. (2011) reported that red wine had a greater protective effect on lipid profile than other alcoholic beverages (Chiva-Blanch et al. 2011). It is also important to note that there is wide variation in the flavonol content of different red wines produced in different regions throughout the world. It seems that sunlight on grapes during cultivation is one factor which determines the flavonol content. This could explain why in a study, Israeli red wine had higher antioxidant capability than UK red wine (Howard et al. 2002). While red wine has been said to be beneficial in the Mediterranean diet, more recently small quantities of any alcohol have been suggested as beneficial particularly for cardiovascular outcomes.

Estruch et al. (2004) explored the anti-inflammatory effects of different alcoholic drinks on markers of atherosclerosis. They found that both wine and gin showed anti-inflammatory effects by reducing plasma fibrinogen (wine by 9% and gin by 5%,) and IL-1 levels (by 21% and 23%, respectively). Red wine had an additional effect, of decreasing hs-CRP a marker of inflammation (Estruch et al. 2004).

Salt

Brown et al. (2009) suggest the salt content of a Western diet of processed foods is high when compared with the Mediterranean diet. Salt intake varies considerably around the world and while the physiological need is about 10–20 mmol/day, in some populations such as in the Western World and Japan the amount of salt consumed is in excess of 200 mmol/day. Processed foods and 'fast food' eaten regularly in the Western World can be more than 100 times higher in salt than that in homemade meals (Brown Tzoulaki Candeias and Elliott 2009) and this has been suggested as a plausible reason for an increase in the prevalence of multiple sclerosis over the last 20-30 years (Manzel et al. 2014). Salt has been shown to modulate the differentiation of human interleukin-17 (IL-17)-producing helper T cells (Th17 cells). These cells are highly proinflammatory (Wu et al. 2013). Since multiple sclerosis is an inflammatory state and the Th17 immune response could be important for multiple sclerosis onset (Kostic et al.2014) it is understandable that salt has been studied in relation to multiple sclerosis aetiology. When salt intake is decreased, this is accompanied by reduced production of proinflammatory cytokines such as interleukin (IL)-6 and IL-23, and upregulation of the anti-inflammatory cytokine IL-10 (Yi et al.2015). Salt intake is modifiable, therefore if high levels of dietary salt were found to be a risk factor for the aetiology multiple sclerosis it would be a useful preventative measure to reduce salt intake. The only human study to associate high levels of salt with multiple sclerosis was the work of Farez et al. (2014). This was an observational study (of disease progression) that measured sodium chloride levels in urine. Since 80-90% of salt ingested is excreted in urine, then urine levels give an approximation of ingested salt levels. However, Ascherio, and Munger (2016) suggest that the evidence so far, is insufficient to draw conclusions. They say only one study has implicated high salt intake with an increased risk of multiple sclerosis and as yet, this has not been confirmed by other studies. In summary, as yet, there are no human studies to suggest salt is associated with the aetiology of multiple sclerosis.

2.3 Potential Confounders

Confounders have been defined earlier in the chapter, and confounding as a type of systematic error is discussed in detail in the methods chapter. It is generally considered that sex, smoking, alcohol, obesity/BMI, and socioeconomic status could be confounding variables and distort the association between the exposure and the outcome.

In order for sex/gender to be a confounder, women would have to eat and drink differently to men. This is highly likely. Mattioli et al. (2013) suggest women showed a higher adherence to the Mediterranean diet than men and León-Munoz et al. (2012) found that men drank significantly more alcohol than women. Consequently, adjustments were made statistically to the results of this study to test if sex was a confounder.

2.3.1 *Sex differences in multiple sclerosis*

At the beginning of the twentieth century it was thought that men were more likely to develop multiple sclerosis than women (Brain 1930). This assumption has been disputed, and there are theories to suggest why this may have occurred. It may well have been that as the breadwinners of the family, men were considered more important for diagnosis, and so numerically, more men received more of the trained neurologist's time. Another possibility is that misdiagnosis of women was more common in that era. Hysteria was often diagnosed for a range of disorders and this may well have been the case in women with multiple sclerosis (Magyari 2014). During the 1940s the sex ratio for the prevalence of multiple sclerosis started to reverse, and studies were published that described a ratio of 1:1 (Talley 2005). Today, it is widely accepted that the prevalence of multiple sclerosis is higher amongst women than men (Orton et al. 2006; Alonso and Hernán 2008; Pugliatti et al. 2009; Libert, Dejager, and Pinheiro 2010; Greer and McCombe 2011; Nussinovitch and Shoenfeld 2012; Golden and Voskuhl 2017; Waubant 2018). The female to male sex ratio by year of birth has been increasing for at least 50 years and now exceeds 3·2:1 in Canada ($p<0.0001$) (Orton et al. 2008).

In their systematic review, Alonso and Hernán found that the female-to-male ratio in multiple sclerosis incidence had increased over time. They estimated that in 1955 the ratio was 1.4:1 but this had risen to 2.3:1 by 2000 (Alonso and Hernán 2008). The increase in the female-to-male ratio has been said to be due to the increased incidence in women, and not by a decreased incidence in men (Golden and Voskuhl 2017). One theory to explain the increased incidence reported in women was the use of the oral contraceptive pill. However, using data from The Royal College of General Practitioners' Oral Contraception Study, Thorogood and Hannaford (1998) found there was not a

greatly elevated risk of multiple sclerosis during or after use of combined oral contraceptives.

In a Canadian study involving 5493 multiple sclerosis patients, women with multiple sclerosis had earlier menarche than their controls, while men with multiple sclerosis and their controls reached puberty at the same time (Ramagopalan et al. 2009). This raises questions about a hormonal influence on development of the disease and perhaps protective effects of male hormones. However, there are some contradictory findings with regard to hormones. Nulliparity, and delayed childbearing, seem to increase the risk of multiple sclerosis (Bove and Chitnis 2014). Whereas, an Iranian case-control study of 399 cases and 541 controls found a protective association between multiple sclerosis and older age at menarche (OR = 0.90 95% CI = 0.82–0.98), older maternal age at first childbirth (OR = 0.94 95% CI = 0.89 − 0.99) and higher number of parities (OR = 0.61 95% CI = 0.49 − 0.75) (Salehi et al. 2018).

In paediatric multiple sclerosis, two peaks of the disease occur. One at 4 years of age and the other at 15 years of age. The sex ratio was 1:1 in the 4year olds but had shifted to a female dominance at 9 years of age. Puberty typically starts between 9 and 14 years of age in boys and 8 and 12 years of age in girls (Waubant 2018). It has been suggested that multiple sclerosis onset may be related to hormonal changes associated with puberty and the age of puberty has tended to decrease over time (Waubant 2018).

2.3.2 *Obesity and multiple sclerosis and the Mediterranean diet*

In order to be a confounder, obese people would have to eat differently to non-obese people. This is quite likely. In addition to confounding, obesity must be considered in relation to reverse causation. Patients with multiple sclerosis may become less mobile as the disease progresses, which may lead to weight gain. It is possible that comfort eating as a response to multiple sclerosis could lead to over-eating and weight gain.

Body Mass Index and Body Mass Index categories

It is widely accepted that obesity is associated with a low-grade inflammatory state Calder et al. (2011), and obesity is a recognised risk factor for multiple sclerosis (Vimaleswaran et al. 2013; Gianfrancesco et al. 2017; Olsson Barcellos and Alfredsson 2017; Correale Farez and Gaitán 2017; Alfredsson and Olsson 2018). A high level of evidence from meta-analysis by Liu et al. (2016) supports this. In order to assess the effect of reverse causation, it would be necessary to know the level of obesity in multiple sclerosis patients prior to their diagnosis. Jelinek (2017) suggests that many of the

observational studies associating obesity and multiple sclerosis have not adequately accounted for confounding or reverse causation.

Body Mass Index (BMI) is the most common method of measuring obesity in adults because it is cheap and easy to calculate. Munger et al. (2009) used data from 121,700 women in the Nurses' Health Study and 116,671 women from the Nurses' Health Study II. Weight and height at 18 years old was used to determine BMI. They found that obesity (BMI >30 kg/m^2) was associated with a two-fold increased risk of multiple sclerosis (Munger et al.2009). It might be suggested that this and other case-control studies on obesity are influenced by bias which could potentially be introduced by confounding and reverse causation (Mokry et al. 2016). The key question is whether the BMI was high before diagnosis or after it. This has been addressed by Hedström Olsson and Alfredsson (2012) who conducted a case-control study in Sweden of 1571 multiple sclerosis cases and 3371 controls. The authors found that those subjects whose BMI exceeded 27 kg/ at age 20 years of age had a two-fold increased risk of developing multiple sclerosis compared with normal weight subjects. The conclusion was that BMI during adolescence, and not childhood, predicted multiple sclerosis risk. The questionnaire in this study did not ask about childhood BMI, but in the medical history section a question about diagnosis of overweight and obesity was asked, and if so a date of diagnosis. In this way it was possible to determine if obesity was diagnosed in childhood, adolescence or adulthood. A further issue that might be raised is recall bias and the validity of weight reporting. The authors address this citing the Nurses' Health Study (Munger et al. 2009), which found high correlations between recalled and measured past weight. In the Nurses' Health Study weight was slightly under-reported especially weight at 18 years of age, with a mean difference of 1.4 kg. Of course, one might expect nurses to be better at reporting their weight than compared to the rest of the population. The suggestion is that under-reporting of weight probably does not differ significantly between cases and controls (Hedström Olsson and Alfredsson 2012). Langer-Gould et al. (2013) undertook a cohort study to research clinically isolated syndrome (CIS) in children enrolled in the KPSC Children's Health Study between 2007 and 2009. Body mass index had been obtained prior to symptom onset. There were 920,034 (89.2% of eligible patients) who had at least one valid weight and height in the 3-year study period. Apart from the lowest and highest ends of the socioeconomic spectrum, the KPSC paediatric membership was representative of the general paediatric population in Southern California with respect to ethnicity, age, gender, and socioeconomic status. The definitions for overweight and

obesity in children and adolescents were based on the sex-specific BMI-for-age growth charts developed by the Centres for Disease Control and Prevention and WHO definitions for overweight and obesity in adults. After exclusions, 913,172 patients were included in the final analytical cohort. Results showed 75 children had newly diagnosed CIS. Thirty-eight (50.7%) children and adolescents with CIS were overweight or obese. After statistical analysis they concluded that childhood obesity was independently associated with a significantly increased risk of CIS in girls but not boys. An adjusted odds ratio of 1.58 was found in overweight girls compared to normal and an adjusted odds ratio of 3.76 in extremely obese girls. The authors postulate that high levels of oestrogen coupled with low grade inflammation released by adipose tissue in the obese may accelerate CIS to multiple sclerosis onset. The suggestion is that obese boys never reach the androgen levels of the girls. Pinhas-Hamiel et al. (2015) compared 130 adult disabled multiple sclerosis patients to the general population. They found disabled multiple sclerosis patients had lower rates of obesity assessed by BMI at 18.5%. However, these patients had higher rates of increased waist circumference and 56% had central obesity determined by waist circumference despite having a lower BMI. A meta-analysis by Liu et al. (2016) examined 5 observational studies including 3 case-controls and 2 cohort studies. The relative risk (pooled) for overweight and obesity during childhood and adolescence when compared with normal weight (body mass index = 18.5-24.9 kg/m^2) was 1.44 (95% CI 1.22-1.70) and 2.01 (95% CI 1.63-2.48), respectively. The conclusion was that higher BMI in childhood and adolescence leads to an increased risk of multiple sclerosis. Severe obesity was an even stronger risk. These results were statistically significant in females, but not in the males.

As stated earlier, confounding and reverse causation must be kept in mind when considering any association between obesity and multiple sclerosis. In the results section, obesity as a confounder is tested statistically.

2.4 Smoking and multiple sclerosis and the Mediterranean diet

In order to be a confounder, smokers or previous smokers would have to eat differently to never smokers. This is possible, since smoking is an unhealthy life choice and someone making an unhealthy life choice might also make an unhealthy eating choice or be less likely to make a healthy diet choice such as those in the Mediterranean diet.

2.4.1 *Evidence of smoking as a risk factor for multiple sclerosis*

For many years smoking has been suggested as a risk factor for development of multiple

sclerosis (Antonovsky et al. 1965; Villard-Mackintosh and Vessey 1993; Ramanujam et al. 2015; Gross and Lublin 2017). Much of the evidence comes from large cohort studies such as The Oxford Family Planning Association Study.

The Nurses' Health Study of American women showed a 60% greater multiple sclerosis rate among current smokers than among individuals who never smoked. The study also showed that the multiple sclerosis rate increased with cumulative smoking exposure, with the greatest risk (1.7 times) among smokers who had smoked for 25 years or more. The large prospective design of these studies confirms that smoking exposure was evaluated prior to a multiple sclerosis diagnosis, thus establishing a temporal relationship.

A metanalysis by Hawkes et al. (2007) stated that six case-control studies had shown similar results to the cohort studies mentioned earlier, that is, the risk of multiple sclerosis is increased for those who smoked prior to disease onset. The increased odds ratios, ranged from 1.22 95% CI: 1.04,1.48 to 1.51 95% CI: 1.22,1.87, depending on the method of analysis. A summary of selected studies and their main outcomes appear in Table 3.

Table 3 Selected studies of smoking and multiple sclerosis 95 % CI 1.35–1.63

Study	Population size and type	Main outcome
The Oxford Family Planning Association Study Villard-Mackintosh and Vessey (1993).	Prospective study of more than 17,000 British women, 63 multiple sclerosis cases.	The risk of multiple sclerosis for women smoking 15 or more cigarettes per day was almost twice that of women who had never smoked relative risk (RR) 1.8 95% CI: 95% CI: 0.9,2.2
The Royal College of General Practitioners' Oral Contraception Study Thorogood and Hannaford (1998)	Prospective study of 46,000 British women.	The risk of multiple sclerosis for women smoking 15 or more cigarettes per day was (RR) 1.4 95% CI: 0.9,2.2,
The Nurses' Health Study I and II Hernan Oleky and Ascherio (2001).	121,700 women and 116,671 US women	The relative incidence rate for current smokers was 1.6 95% CI: 1.2,2.1, and 1.2 95% CI: 0.9,1.6 for past smokers
A case-control study in Sweden Hedström et al. (2013).	1,798 cases and 3,907 controls	Smoking was related to an increased risk of multiple sclerosis and in a dose related manner. Compared with never smokers, 'ever smokers' had an increased risk of developing multiple sclerosis OR 1.5 95% CI: 1.35,1.63,

2.4.2 *Passive /second hand smoking and multiple sclerosis*

Passive smoking has been associated with almost all the diseases associated with smoking as the toxic chemicals found in smoking are also found in second hand smoke.

In an overview of systematic reviews Cao et al. (2015) highlight the difficulties in measuring passive smoking. The type of tobacco smoked, and the volatility of the agents make comparisons difficult (Kritz Schmid and Sinzinger 1995).

Metabolites of tobacco have consistently been used as biomarkers for passive smoking. Measurements may be made in blood, urine, saliva, breast milk, amniotic fluid, hair, and teeth (Winstanley 2008). The most commonly used biomarker is cotinine, which is a major metabolite of nicotine. It is sensitive enough to distinguish between people not exposed to second-hand smoke and those exposed to low, moderate and high levels of

second-hand smoke. The half-life of cotinine is around 20 hours, so cotinine levels reflect exposure to tobacco smoke in the preceding one or two days. Nicotine can be analysed from hair samples and can indicate exposure over a period of months (Winstanley 2008). Alternatively, the measurement of urinary trans-muconic acid (Taniguchi et al. 1999) or expired carbon monoxide (CO) (Belabbaci et al. 2016) have been used.

A summary of selected studies and their main outcomes appear in Table 4.

Table 4 Selected studies of smoking and passive smoking and the association with multiple sclerosis

Study	Population size and type	Main outcome
Hernan et al. (2001)	The Nurses 1 study of 121,700 women and The Nurses II of 116,671	The relative rate increased significantly with cumulative exposure to smoking. Compared with that for women who never smoked, the relative incidence rate of multiple sclerosis was 1.6 (95% CI: 1.2, 2.1) among current smokers and 1.2 (95% CI: 0.9, 1.6) among past smokers after adjustment for age, latitude, and ancestry. The relative rate increased significantly with cumulative exposure to smoking (p for trend < 0.05), from 1.1 (95% confidence interval: 0.8, 1.6) for 1-9 pack-years to 1.5 (95% CI: 1.2, 2.1) for 10-24 pack-years and 1.7 (95% CI: 1.2, 2.4) for 25 or more pack-years
Ghadirian et al. (2001)	197 multiple sclerosis cases and 202 controls	'Ever-smokers' 20-40 cigarettes per day an odds ratio (OR) of 1.9 (95% CI: 1.2, 3.2) Heavy smokers-40 per day the odds ratio was OR 5.5; 95% CI: 1.7, 17.8)
Sundström et al. (2008)	Cotinine levels of 109 multiple sclerosis cases and 218 matched controls.	Elevated cotinine was associated with an increased risk for multiple sclerosis

		OR=3.9; 95% CI: 1.3–12
Hawkes et al. (2007)	Metanalysis	Increased odds ratios, ranged from 1.22 95% CI: 1.04,1.48 to 1.51 95% CI: 1.22,1.87
Hedström et al. (2011)	695 cases, 1635 controls	Never-smokers exposed to passive smoking had an increased risk OR 1.3, 95% CI 1.1-1.6
Abdollahpour et al. (2017)	547 cases and 1057 controls	Passive smoking was significantly associated with multiple sclerosis OR = 1.85 (1.48–2.32)

The data have been inconsistent regarding the influence of passive smoking on multiple sclerosis. An extreme example is the effect of maternal smoking on the foetus during pregnancy which showed no risk of multiple sclerosis risk in the offspring (Montgomery et al. 2008; Ramagopalan et al. 2013). On the other hand, Zhang et al. (2016) undertook a meta-analysis based on 20,626 cases from case-control and cohort studies. They included four study populations that excluded active smokers and the pooled OR was 1.24 (95% CI [1.03–1.49], p = 0.028), indicating that exposure to passive smoking increases the risk of multiple sclerosis by 24% compared with unexposed individuals.

It has been estimated that the risk of multiple sclerosis is increased by approximately 50% in cigarette smokers (Handel et al. 2011; Amato et al. 2017). And this risk has been said to increase in a dose-related manner (Kamm Uitdehaag and Polman 2014; McKay et al. 2016). Smoking and passive smoking are said to pose a considerable and preventable risk of acquiring multiple sclerosis (Hedström et al. 2017). Indeed, the suggestion is that in Sweden about 20% of all multiple sclerosis cases are attributable to active or passive smoking (Hedström et al. 2017).

2.4.3 *Smoking in Cyprus*

The association between smoking and multiple sclerosis is strong in observational studies, but no studies were found for Cyprus.

There are large numbers of Cypriots who smoke. Eurostat (2014), suggests that 25.2% of Cypriots smoked in 2014. Only Bulgaria 27.3%, Turkey 27.2%, Greece 27% and Hungary 25.8% had a higher percentage of the population that smoked.

These data are similar to those found by Zinonos (2016) who suggest that 26.5% of the

Cypriot population smoke. In a study of healthcare staff at Nicosia General Hospital the figure was slightly higher. Of 119 doctors and 392 nurses questioned, 28.6% of the doctors and 28.1% of the nurses said they currently smoked (Zinonos et al. 2016). By comparison, a study of 600 participants published in 2015 found that 37.7% (n=220) had never smoked, 19% (n=114) were former smokers, 9.2% (n=55) were occasional smokers and 35% (n=211) were daily smokers (Lazuras et al. 2015). The figure of 35% for daily smokers is 10% higher than the Eurostat estimate and amounts to over 1/3 of the adult Cypriot population. Cooter et al. (2015) found that the percentage of Cypriot males who smoked daily had remained around 38% between 2003 and 2008 (down from 43% in 1989) and this percentage was similar in 2014 at 37.3% (Eurostat 2014). However, females who smoked daily had risen from 7% in 1989 to 10.5% in 2003 and then 14.3% in 2008 (Cooter et al.2015; Farazi et al.2015). It remains around 14% according to the 2014 data (Eurostat 2014) but there is no explanation why smoking among women doubled between 1989 and 2008. Nicolaou et al. (2016) used data from the 2009 Countrywide Integrated Non-communicable Disease Intervention questionnaire to assess smoking in the Greek Cypriot population. There were 3,021 young and middle-aged adults (25-64 years old) in the survey. It was found that 50.8 % of adult men and 21.2 % of adult women currently smoked, and that just around 50% of the sample either currently smoked or had ever smoked. Since large numbers of Cypriots smoke (over 25%), it is not surprising that many teenagers are subjected to passive smoking. This could be a major risk factor in acquiring multiple sclerosis.

Multiple sclerosis is approximately twice as common in women than men and has increased in prevalence in Cyprus from estimates by Middleton and Dean (1991) of 44.5 per 100 000 to 198 per 100,000 (Charalambidou Pantzaris and Patrikios 2016). A partial explanation could be the rise in smoking among women.

Smoking amongst Cypriot teenagers is particularly alarming. Cypriot researchers defined current smoking, as having smoked cigarettes on one or more days of the past 30 days. When 7,294 Middlle school children (12-15year-olds) were surveyed it was found that 13% of boys and 7% of girls had smoked. When 5,952 high schools (15-18-year-olds) teenagers were surveyed, the figures rose to 36% among boys and 23% among girls. (Christophi et al. 2008: Christophi et al. 2016). This is in contrast to a 2003 study which found 60% of high school male teenagers had ever smoked but only 25% of males had smoked in the past 30 days (Bjarnason et al. 2003). The rise of 11% over this 13year time period is in contrast with the decline seen in some other countries. What is also

interesting is that in 2003 only 9% of high school girls were smokers but by 2016 this had risen to 26%. This is almost a three-fold increase. Further data collection in 2016 on passive smoking, provided even more worrying results. More than half of the students (53.5%) had at least one parent that smoked, and 84.6% lived in homes where others smoked around them. Outside the home, 91% said they were present among other smokers (Christophi et al. 2008).

Between a quarter and a third of Cypriots smoke and most of the regular smokers, smoke cigarettes. Many more men smoke than women, but while the male numbers have remained similar over a 19year period the number of women smokers has doubled.

The number of children and adolescents who smoke is high and seems to be increasing, especially among girls. It is well- known that teenage smokers progress to adult smokers, so this is worrying for future healthcare needs. If smoking does indeed predispose people to multiple sclerosis, then the prevalence of multiple sclerosis is likely to increase if smoking among Cypriots increases.

2.5 Socioeconomic status and multiple sclerosis and the Mediterranean diet

In order to be a confounder, people from one socioeconomic group would have to eat differently to those from another. This is highly plausible, and results were tested statistically to detect confounding.

Zilber and Kahana (1996) conducted a case-control study which included 93 cases and 94 matched controls. The study found patients and their parents had higher educational levels than controls ($p < 0.01$) and concluded that less-advantaged socioeconomic status were protective against multiple sclerosis, but this observation was only statistically significant when living conditions are well below average. A case-control study conducted by Kurtzke and Page (1997) recruited 5,305 US veterans. Multiple sclerosis cases totalled 80 and 77 were included in the study and matched with controls. The socioeconomic data was obtained from military records therefore eliminating recall bias. The authors found that higher socioeconomic status was associated with higher risk of multiple sclerosis in black and white men and white women (Kurtzke and Page 1997). The sub group of black military women with multiple sclerosis was very small and this may account for them not having a similar association with socioeconomic status to the men.

There have been several problems with regard to socioeconomic status (SES) association and multiple sclerosis over the last 50 or 60 years. First of all is the definition of

socioeconomic status which varies considerably from study to study. One definition includes the summation of income, education and occupation (Bradley and Corwyn 2002). The MacArthur Scale of Subjective Social Status was developed in order to make sense of a person's view of their own position on social ladder (Adler and Stewart 2007). Obese people have been shown to have an increased risk of multiple sclerosis and obesity is associated with lower SES (Mayor 2017) so confounding is likely. There was no strong association between SES and multiple sclerosis in a Danish national register study. However, there was a tendency towards a reduced risk of multiple sclerosis among children of mothers with higher education (Nielsen et al. 2013). This is contrary to earlier published data. In a review published in 2015 Berg-Hansen and Celius state that there is still controversy as to whether the risk of multiple sclerosis is affected by socio-economic status (SES) (Berg-Hansen and Celius 2015). A systematic review published in 2015 found that there were inconsistent associations between high SES and increased multiple sclerosis risk. The authors concluded that most studies failed to control for other important risk factors for multiple sclerosis (Goulden Ibrahim and Wolfson 2015). Further work by Goulden et al. (2016) found that when adjusting for other risk factors and taking three countries into account (Norway, Canada and Italy) there was no consistent association between parental SES and multiple sclerosis risk, except a protective effect of low SES only found only in Canada. Bjørnevik et al. (2016) analysed 4494 multiple sclerosis patients in a Norwegian registry born between 1930 and 1979. The level of education was found to be inversely associated with multiple sclerosis risk (p trend < 0.001) with an odds ratio (OR) of 0.73 (95% confidence interval (CI): 0.59–0.90) when comparing the highest and lowest levels. The conclusion was that the level of education was inversely associated with multiple sclerosis risk. Further work by Bjørnevik examined education levels in 953 multiple sclerosis patients and 1717 healthy controls from Norway. Higher levels of education are associated with decreased multiple sclerosis risk (p trend = 0.001) with an OR of 0.53 (95% CI 0.41–0.68) when comparing those with the highest and lowest level of education. The risk was slightly reduced when adjusted for other risk factors such as smoking.

Smokers have been shown to have an increased risk of multiple sclerosis, and there are more smokers in lower social groups thus confounding the effects of SES on multiple sclerosis (Hiscock et al. 2012). Bjørnevik et al. (2016). concluded that a higher level of education was associated with lower multiple sclerosis risk. A healthy diet has been associated with higher socio-economic status (Tong et al. 2018) which may in part be

explained by more disposable income in this group of people. However, this is not true in all populations. For example, Yau et al.2019 found diet quality varied across ethnic groups and low socioeconomic status was not consistently associated with poor diet quality.

If SES is associated with the risk of multiple sclerosis, (I do not think there is enough consistent evidence to say it is) there are several theoretical mechanisms. SES and diet are related (lower SES is often but not always associated with poorer diet). Diet and obesity are related, (poorer diet may be associated with increased obesity). SES and obesity are related, lower SES may be related to poorer diet and hence higher obesity. SES is related to smoking habits (lower SES populations are more likely to smoke) and so is possible smoking confounds both the associations of SES with diet and obesity. Any associations of SES and the risk of acquiring multiple sclerosis is potentially confounded by smoking.

Other risk factors for multiple sclerosis were considered but deemed unlikely to be confounders in this study. These included: skin type and sunlight exposure, latitude, vitamin D deficiency, month of year born, infections, inflammatory diseases and genetic factors.

Less likely associations: Lack of exercise, previous surgeries, stress and periodontal disease.

Debunked theories have included: Amalgam fillings, MMR and other vaccinations and geographical clusters.

Summary and appraisal of evidence

It is widely accepted that genetics accounts for 15-30% of the aetiology of multiple sclerosis (Wang et al. 2016), and environmental triggers combined with this genetic susceptibility most likely initiate the disease in late teenage years and the early years of adulthood. Migration studies have supported the notion that multiple sclerosis is due more to environmental exposure than genetics (Kurtzke 2013). A latent period exists, and disease diagnosis occurs sometime after exposure. Many risks factors have been proposed over the years and although there are strong candidates, causative agents remain elusive. Evidence of association has mainly been from observational studies and in some studies the results are contradictory. While the quality of other studies is on occasions low.

<u>Viruses</u>

There is strong evidence for viral triggers especially EBV. Using serology testing, almost

all multiple sclerosis cases show past exposure to EBV. The past medical history section of the questionnaire therefore asked questions about previous viral infections.

Smoking

Smoking is an established risk factor for multiple sclerosis (Hedstrom,Hillert and Olsson 2013). Large cohort studies and meta-analysis support the view that smoking prior to disease onset increased the risk of multiple sclerosis. Hawkes (2007) suggests the OR ranged from 1.22 to 1.51.

In the current study smoking is a potential confounder and needs to be statistically tested and interpreted in the results.

Sex/gender

Initially, men were affected more than women, but the sex ratio has changed over the decades and it is now accepted that women are affected about 3 time more than men, (Waubant 2018). Sex/gender is a potential confounder in the current study.

Vitamin D deficiency

For many years vitamin D deficiency has been proposed as a risk factor for multiple sclerosis. There is strong evidence for this association, supported by several large cohort studies including that of Munger et al. (2016) and meta-analysis (Duan et al.2014). Questions on vitamin D deficiency were included in the questionnaire of this current study as it was a potential confounder.

Socioeconomic status

Buchter Dunkel and Li (2012) proposed that the risk of multiple sclerosis could be related to lifestyle and purchasing power. Those of a higher high socioeconomic status being potentially more at risk. Goulden Ibrahim and Wolfson (2014) state high socioeconomic status (SES) is generally associated with better health outcomes but their systematic review found any risk of high SES and multiple sclerosis to be inconsistent. Abdollahpour et al. (2018) found no association between socioeconomic status and risk of multiple sclerosis.

It has not been possible draw any conclusions from the literature review and the association of SES and multiple sclerosis seems to be inconsistent. Perhaps the confounding effect of higher smoking levels in lower socioeconomic groups is a factor.

Diet and the gut microbiota

Substantial evidence links diet and the gut microbiota (Szychlinska et al. 2019). It has been suggested that the gut biome of multiple sclerosis patients is different to the rest of the population (Kirby and Ochoa-Repáraz 2018). It is possible that the gut microbiome

can interact with the central nervous system through bidirectional signaling (Carabotti et al. 2015). While the theoretical link between diet and the risk of acquiring multiple sclerosis via changes to the biome is plausible, few studies have addressed this. Many dietary studies implicate vitamin D deficiency as a risk for multiple sclerosis. There is a paucity of studies in this area of research., whilst there are several reviews and animal studies. The following two studies are closely linked to the current study:
Ghazavi et al. (2019) conducted an age-match case control study in Iran and found a higher intake of processed meat OR 2.07 (95% CI:(1.18-3.63) and non-processed meat OR1.38 (1.13-1.68)) were found in the cases. Sedaghat et al. (2016) found in a case control study (69 case and 140 controls) a Mediterranean type diet was less likely to be consumed by multiple sclerosis cases.

Alcohol

Consuming alcohol has been suggested as both a risk factor (Pekmezovic et al. 2006) and as a protective factor for multiple sclerosis (Hedström Hillert Olsson and Alfredsson 2014). Wang et al. (2015) reviewed the literature and stated that some cross-sectional studies with large sample size had poor responses to the questions on alcohol. They concluded that there is insufficient information to suggest an association between consuming alcohol and multiple sclerosis.

Salt

While animal studies suggest high levels of salt may be associated with a risk of multiple sclerosis, there are no human studies to support this association.

Skin type and sunlight exposure

Skin type and sunlight exposure have both been proposed as risk factors for acquiring multiple sclerosis, but neither are likely to affect diet in cases or controls.

Latitude

Several meta-analyses support the view that a latitude gradient of multiple sclerosis incidence in Europe exists (Wang Simpson and Taylor 2018) but this may reflect vitamin D levels which are affected by seasonal changes in sunlight exposure (Longo et al. 2018). Other studies have suggested this latitude gradient has declined for example in Norway where Grytten Torkildsen and Myhr (2015) suggest there is no longer evidence of a latitude gradient.

The latitude effect is academic in respect to the current study as all the participants are from the same latitude.

Lack of exercise, previous surgeries, stress and periodontal disease

There is little or no evidence to support these theories

Debunked theories have included: Amalgam fillings, MMR and other vaccinations and geographical clusters.

To recap, the association between diet, or a Mediterranean type diet and the aetiology of multiple sclerosis is limited. Few studies have examined this association. However, there are many diet studies, and some Mediterranean diet studies suggesting an association of diet and cardiovascular disease. To a lesser extent, there is a diet association to colorectal cancer.

This study aims to full some of the gaps in the current knowledge.

Chapter 3 Aims and objectives

Aims

The *primary aim* of this study was to investigate the relationship between a Mediterranean dietary pattern and multiple sclerosis in a European population with a higher prevalence of the disease than countries of a similar latitude.

Secondary aims were to investigate the relationship between dietary intake of specific food groups (e.g. fruits, vegetables, alcohol) and individual foods (e.g. tomatoes, citrus fruit) and multiple sclerosis in this population.

The *Null hypothesis tested* was that there is no difference in Mediterranean diet pattern score, or intake of specific food groups, or foods, between multiple sclerosis cases and control participants.

The *objectives* were:

- To design and carry out a population-based case-control study in the Republic of Cyprus, which has a higher than average prevalence of multiple sclerosis in the general population.
- To measure diet and socio-demographic factors in patients attending for treatment of multiple sclerosis compared with “healthy”, age and gender-matched control subjects.
- To assign a Mediterranean diet score to the recorded diets and compare this score between multiple sclerosis patients and age-matched controls.

- To identify specific food groups and individual foods characteristic of the Mediterranean diet and compare intake of these foods between multiple sclerosis patients and age-matched controls.
- To investigate the impact of socio-demographic factors on these relationships.
- To explore if multiple sclerosis cases avoided any foods.

Chapter 4. Methods

This chapter deals with the methods used to address the aims and objectives of the study outlined in Chapter 3. Many authors of case-control studies follow the 22point STROBE Checklist because it is considered the gold standard for this type of study (Lynch Popchak and Irrgang 2019). STROBE stands for STrengthening the Reporting of OBservational studies in Epidemiology; and was adopted for this study. The STROBE Checklist is shown in Appendix B. This chapter begins with the study design and setting. It then explains the selection of cases and controls for the logistic regression analysis and then describes the selection of cases and controls for the age-matched conditional regression analysis. Variables and potential confounders are addressed next. Finally, data sources, bias, study size, analysis of quantitative variables and statistical methods are described.

4.1 Ethics approval

The study was completed in compliance with the Declaration of Helsinki for studies involving human volunteers. Study recruitment did not commence until Ethical approval was obtained. Ethical approval was provided by the University of Newcastle upon Tyne Faculty of Medical Sciences Research Ethics Committee, reference number 0 1 2 6 9 granted on 15/5/17 (Appendix E 2). Local Ethical approval was provided by the Cyprus National Bioethics Committee, reference number 0984669 (Appendix E 1).

All study literature was provided in Greek and English and all volunteers gave written informed consent after having the opportunity to ask questions of the research team. Participants were told that they could withdraw at any stage of completing the questionnaire without needing to provide an explanation and without compromising any aspect of their treatment.

4.2 The study design

This observational study was a population-based case-control study. The study population was restricted to the Cypriot population of The Republic of Cyprus, since the Turkish occupied third of the island posed language and ethics challenges to the study beyond the time-frame available. Exposure to the variable of interest was recorded in both cases and controls and any association exposure and disease assessed.

As stated in chapter 1, multiple sclerosis is a rare disease which is most likely initiated by a trigger or triggers in genetically susceptible people and is often diagnosed some years after exposure. A case-control study is a useful study method for a rare outcome or for an

outcome that may have a long latent period. A case-control study can be used to study multiple exposures, and associations can be made between exposure and outcome. Case-control studies are relatively fast to complete, especially compared with cohort studies.

4.2.1 *Participant Inclusion criteria*

Cases and controls were Greek Cypriots recruited from the general population from the Republic of Cyprus. Any person >18yrs old with diagnosed multiple sclerosis or a healthy person was eligible to take part. The study purpose and requirements were described to potential participants who also read the study aims (Appendix C). If participants agreed to take part, they signed an informed consent form before entering the study (Appendix C)

Defining a "Greek Cypriot" proved rather difficult in practice. A "Greek Cypriot" is a social construct and anyone who considered themselves a "Greek Cypriot" was deemed a "Greek Cypriot". Anyone born in The Republic of Cyprus was also classed a "Greek Cypriot" if their parents deemed themselves a "Greek Cypriot".

Some people who met the study definition of "Greek Cypriot" chose to consider themselves something other than "Greek Cypriot" and entered that in the ethnicity section of the questionnaire.

4.2.2 *Participant Exclusion Criteria*

Any potential case or control aged under 18 at the time of the study was excluded. Anyone diagnosed with Clinically Isolated Syndrome (CIS) (pre- multiple sclerosis symptoms, but not a definitive diagnosis) were not regarded as a multiple sclerosis case and were also excluded. Individuals unable to provide informed consent to take part for whatever reason were excluded.

4.3 Recruitment of Cases

Multiple sclerosis cases were defined using recognised diagnostic criteria and selected by convenience sampling due to the small numbers of cases on the island and the timescale of the study. Multiple sclerosis cases were "Greek Cypriot" people aged 18 and over, currently living in The Republic of Cyprus with a confirmed diagnosis of multiple sclerosis. The diagnosis of multiple sclerosis had to have been made by a consultant neurologist using the accepted diagnostic criteria at the time of diagnosis. There was no time limit between diagnosis and the time of taking part in the study.

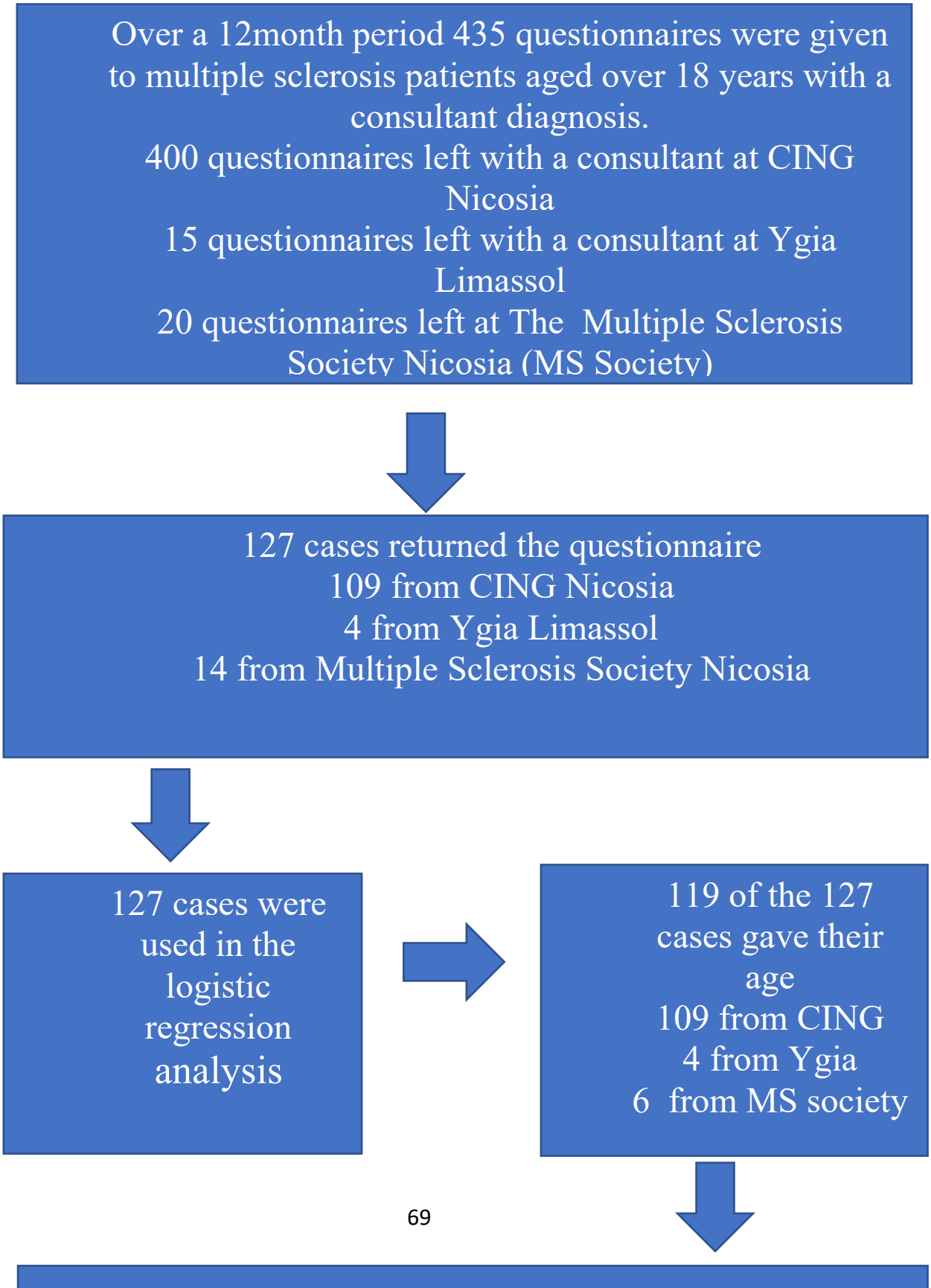
Approximately 435 questionnaires were given to patients at The Cyprus Institute of Neurology and Genetics (CING) when they attended for their review during a one-year period between August 2017 to August 2018. Completed questionnaires were available for 109 cases from this clinic. Some of the self-administered questionnaires n=10 (approximately10%) were completed on the premises, while the patients waited for physiotherapy or another treatment, whereas others were completed at home and brought back to the clinic during a subsequent appointment or when the participant was passing the clinic.

In Limassol, 15 questionnaires were given to patients with multiple sclerosis at The Ygia Polyclinic Private Hospital. Four completed questionnaires were obtained for cases from this source.

It is possible that this "clinic-based" recruitment could exclude those multiple sclerosis cases which were "least disabled" because they did not seek regular treatment and the "most disabled" since they may be house-bound and not able to attend hospital clinics. For this reason, in Nicosia, at the Nicosia Multiple Sclerosis Society headquarters, 20 questionnaires *were* given throughout the year to patients attending for social functions and/or physiotherapy. A total of 14 multiple sclerosis patients were recruited from this location.

All types of multiple sclerosis were regarded as multiple sclerosis and no distinction was made of the various phenotypes. A summary of recruitment of cases is shown in Figure 15

Figure 15 Flow diagram to clarify case recruitment in both analyses



As can be seen, cases were recruited by convenience sampling. While it was attempted to recruit cases from clinics and hospitals across the island, this proved more difficult than anticipated. Most cases came from CING in Nicosia although some cases may have traveled from other towns in Cyprus to CING in Nicosia.

4.4 Recruitment of Controls

Controls were people 18 years and over who did not have a diagnosis of multiple sclerosis. Recruiting hospital-based controls might have been efficient and cheap to undertake, but bias can arise from the illness of the controls. For the same reason, it was decided not to use relatives or spouses of cases as controls. If relatives had been used, there was a risk of "over-matching". These people may have had similar environment exposure as the cases, and their diet might have been so similar that drawing conclusions could be difficult. For example, if a spouse is a control, it is likely they will eat similar if not the same meals as the case. Ideally, the controls would have been randomly sampled using a postal code method or street number chosen randomly. However, the time-scale of the study (2 years from start to finish) and the costs of a postal survey precluded this method.

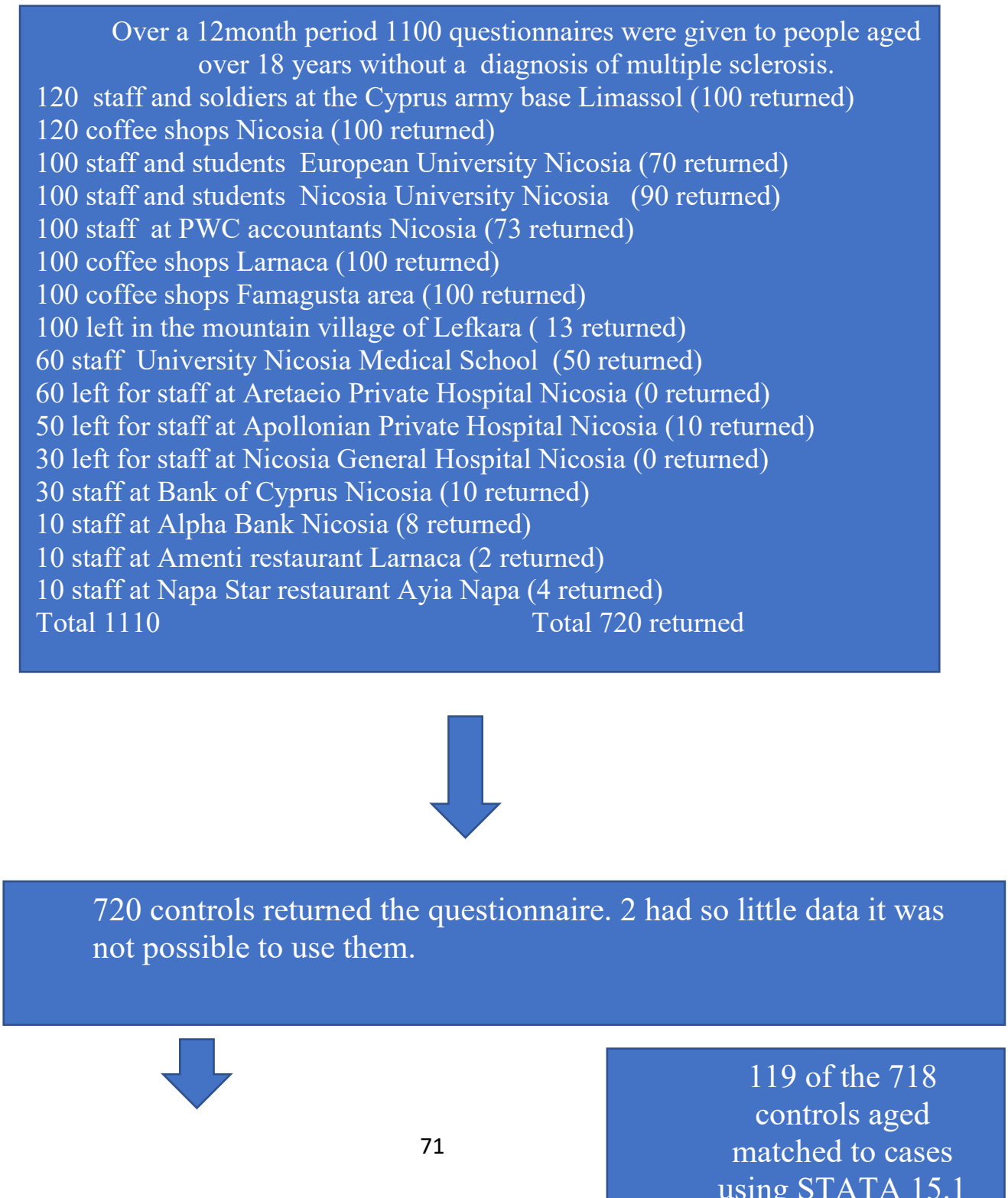
So, controls were recruited by convenience sampling from coffee shops, offices, schools, colleges and family gatherings throughout The Republic of Cyprus.

Approximately 1100 questionnaires were distributed and after 1 year, 720 had been completed (fully or in part) and returned. This represented a response rate of around 65%.

Matching is popular in case-control studies. Matching may be on any potential confounder, for example, age, sex or demographics or a combination of these. Matching usually increases the efficiency of the study but may reduce the power by excluding those cases and controls that cannot be matched. In some case-control studies it is possible to match on both age and sex. Ideally, recruiting cases first would have allowed recruiting matched controls on both age and sex, but this was not possible due to the time restraints of the study. Case recruitment also proved slower than anticipated. As a result, in this study cases and controls were recruited simultaneously. Age-matching was done by matching the 119 cases with age available to 119 controls. All but 7 cases could be

matched exactly by year to one or more controls. The remaining 7 cases were matched to controls who were 1 year above or 1 year below the age of the case. Where more than one control had the same year of birth as a case the control was chosen by generating random numbers and allocating a random number to each control. The highest number was matched with the case of the same age.

Flow diagram to clarify control recruitment in both analyses



As previously stated, control recruitment was by convenience sampling. This method of recruitment has several potential flaws. While it was attempted to recruit from around the island, clearly this was not possible.

Most of the recruitment was from Nicosia. Statistical analysis was used to test whether the demographics of cases and controls were different.

Using mainly working people and students (banks, universities and coffee shops), potential age differences might occur between the 718 controls and the 127 cases in the logistic regression analysis. Indeed, this was the case. This potential bias was eliminated in the age-matched conditional regression analysis.

Potential demographic differences between the cases and controls in both analyses were tested by statistical analysis.

Confounding

Confounding is a type of systematic error which may distort the association between the exposure and the outcome. It may account for all or part of an apparent association or it may cause an overestimate of the true association (positive confounding) or an underestimate of the association (negative confounding). (LaMorte and Sullivan 2014).

According to the Boston University School of Public Health (2013) Three conditions must be present for confounding to occur:

1. The confounding factor must be associated with both the risk factor of interest and the outcome.
2. The confounding factor must be distributed unequally among the groups being compared.
3. A confounder cannot be an intermediary step in the pathway from the exposure of interest to the outcome of interest.

Having met these conditions, the statistical analysis would be expected to produce a difference in association of 10% or more if the variable was a confounder (discussed below).

Matching such as age-matching or sex-matching eliminates each of these potential confounders at the design stage.

In the STROBE "checklist" item 7 suggests "Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable" While item 12 states "Describe all statistical methods, including those used to control for confounding" In the main results item 16 states "Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included".

The more likely confounders in this study included age, sex (gender), obesity/BMI, smoking, alcohol and socioeconomic status. Less likely confounders in this study included skin type and sunlight exposure, latitude, vitamin D deficiency, infections, inflammatory diseases and genetic factors.
Sedgwick (2015), suggests potential confounding can be controlled at the design stage or adjusted during the statistical analysis. He says the former is more efficient than the latter. In this study, latitude and possibly sunlight exposure were removed as confounders at the design stage by undertaking the study in a single setting (Cyprus) with a particular climate. Confounding by other risk factors was adjusted at the analysis stage by using logistic regression (Sedgwick 2013).
The magnitude of confounding in a case-control study can be quantified by computing the percentage difference between the crude and adjusted measures of effect. For example,

The magnitude of confounding = OR (crude) – OR (adjusted) / OR (crude)
Or
The magnitude of confounding = OR (crude) – OR (adjusted) / OR (adjusted)

These methods produce slightly different results, but both provide a reasonable measure of confounding (LaMorte and Sullivan 2014). A cutoff of 10% is commonly used to identify confounders (Lee 2014), but this might not always be appropriate. Lee (2014)

undertook a study to examine cutoffs required under different conditions. It was found cutoff points for the change-in-estimate criterion varied according to the effect size of the exposure–outcome relationship, sample size, standard deviation of the regression error, and exposure–confounder correlation. Despite this, in the current study a cutoff of 10% was used.

4.5 Missing Data

There were some missing data from both cases and controls and is addressed in detail within the individual questionnaire sections. Missing data can be dealt with in several ways. For example, the so-called "Complete case analysis" is where all cases and controls with incomplete data are removed from the study. Had anyone with even minor amounts of data missing in either analysis been totally removed, for example a person who missed out a single question on smoking, there would have been very few cases and controls left. The cases and controls who did not give their age were totally excluded from the analysis in the age-matched analysis. "Single imputation", replaces missing data in a variable with a value that best represents the mechanism of the missing data. For example, if someone says they don't drink wine and then data on red wine are missing, then it is reasonable to replace this value with zero rather than leave it blank. Elsewhere, any missing data in this study were left as blank (null) entries.

4.6 The questionnaire

The whole questionnaire in English is shown in Appendix G. The Food Frequency Questionnaire (FFQ) section was derived from The Epic-Norfolk questionnaire (Day et al. 1999) and the questions were the same as those used in the Newcastle Thousand Families Study (NTFS). The Epic-Norfolk questionnaire has been validated using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers (Bingham et al. 1997). Katsouyanni et al. (1997) evaluated the reproducibility and relative validity of a 190-item semi-quantitative food frequency questionnaire (FFQ) in a Greek setting. They concluded there were significant correlations between diet and independent biochemical markers which corroborates the relative validity of the questionnaire in a Greek population. Unfortunately, there has not yet been a validation of the FFQ in a Cypriot setting, although this is planned. In the current study, the FFQ was adapted with the help of a local expert, Dr Elena Philippou, Associate Professor in Nutrition and Dietetics at the University of Nicosia. For example,

branded foods which are not found in Cyprus were omitted and foods such as souvlaki (not usually found in UK) and local alcoholic drinks such as Zivania were included. The Epic-Norfolk questionnaire had been adapted for the Newcastle Thousand Families Study and this template was used in this study. Questions referred to standard servings of foods consumed in the last year (see section 4.6.1).

The other sections in the questionnaire were also derived from the NTFS which was a longitudinal epidemiology study started by Sir James Spence in May and June of 1947. There were 1142 babies born in those months and although the study was originally planned to follow the babies for a year it continues today (Spence Walton and Miller 1954; Hind et al. 2019). The NTFS questionnaire was first used in a follow-up study in 1997 (Lamont et al. 1998) and again in 2007 (Pearce et al. 2008).

The questionnaire was divided into two sections:

1. Collecting information on the frequency and type of foods consumed using a food frequency questionnaire (FFQ)

2. Collecting socio-demographic information including skin type, smoking, alcohol intake, and general health. (see appendix for questionnaire in English).

Details of each section (apart from skin type) are included below.

Each questionnaire from the cases was given a number and each response was entered in the Excel spreadsheet. The controls were treated similarly. The data from the spreadsheet was then uploaded to the software.

4.6.1 *Diet*

The questions on diet refer to the frequency of consuming a range of foods eaten during the preceding year. Participants were asked to indicate by ticking the box corresponding to their estimate for the frequency they consumed each of the foods listed. Frequency of consumption was scored on a nine-point scale:

Never or < 1 a month, 1 - 3 x a month, 1 per week, 2 - 4 x 1per week, 5 - 6 x a week, daily, 2 - 3 x a day, 4 - 5 x a day, 6 + a day

The kinds of food eaten can be grouped to provide a descriptive or characteristic diet type/pattern. This case-control study focussed on associations between multiple sclerosis and the Mediterranean diet pattern which has been regarded as a healthy dietary plan characterised by higher consumption of plant-based foods such as fruit and vegetables

and whole grains, and lower consumption of dairy products and low consumption of red meat and meat products. Mediterranean diet scores have been developed to assess how close a person's diet is to the Mediterranean diet. Several scores exist, and a Modified Mediterranean Diet Score was developed for use in this study as described below.

4.6.2 *Mediterranean Diet Scores*

The current study used a validated FFQ previously used in the NTFS for a Newcastle population, which was taken from the Epic-Norfolk FFQ which was validated for that study population. No validated FFQ currently exist for Cyprus, although one is in the process of validation (personal communication 2019). Validation of the Epic-Norfolk FFQs came via a complex validation process which used urine analysis, to measure nitrogen excretion and compare it to estimated intake. Although the Epic-Norfolk FFQ was not designed to be used to derive a Mediterranean Diet Score, it was considered that the data could be incorporated into a Modified Mediterranean Diet Score which will be described later.

The MedDietScore developed by Panagiotakos, has been used mainly to assess if adherence to a Mediterranean diet reduces the risk of cardiovascular disease. Panagiotakos et al. used the weekly consumption of 9 food groups plus olive oil and alcohol intake to derive 11 scores as shown in Table 5. The score has been found valid and repeatable in many studies and has over 380 citations in the literature. However, it has not been validated in Cyprus, although it has been validated in Greece which has similar foods to Cyprus.

Table 5 Panagiotakos et al. MedDietScore system (Panagiotakos et al., 2007)

Frequency (servings per week) and allocated score						
1.Non-refined cereals	Never =0	1-6 =1	7-12 =2	13-18 =3	19-31 =4	>32 =5
2.Potatoes	Never =0	1-4=1	5-8=2	9-12=3	13-18=4	>18=5
3.Fruits	Never =0	1-4=1	5-8=2	9-15=3	16-21=4	>22=5
4.Vegetables	Never =0	1-6=1	7-12=2	13-20=3	21-32=4	>33=5
5.Legumes	Never =0	<1=1	1-2=2	3-4=3	5-6=4	>6=5
6.Fish	Never=0	<1=1	1-2=2	3-4=3	5-6=4	<6=5

7.Red Meat and products	<1=5	2-3=4	4-5=3	6-7=2	8-10=1	>10=0
8.Poultry	<3=5	4-5=4	5-6=3	7-8=2	9-10=1	>10=0
9.Full fat dairy products	<10=5	11-15=4	16-20=3	21-28=2	29-30=1	>30=0
10.Olive-oil in cooking (times/week)	Never=0	Rare=1	<1=2	1-3=3	3-5=4	Daily=5
11.Alcohol 10 units=1000ml.	<300=5	300=4	400=3	500=2	600=1	>700=0

Intake of each food is estimated, and a score allocated from 0-5 (or the reverse) allocated to each group according to the position of the food group in the Mediterranean diet pyramid. The total MedDietScore is the addition of each group and ranges from 0-55. People with the highest scores are considered to adhere more closely to the Mediterranean diet. For example, a food such as fruit, that is considered to be typical of the Mediterranean diet, is given a score of zero if fruit is never eaten in a week and 5 if it is eaten more than 32 times in a week.

The converse for a food such as red meat, that is not considered to be typical of the Mediterranean diet, a score of 5 is given if the red meat is never eaten in a week and 0 if it is eaten more than 10 times in a week.

The scoring system is “not perfect, but a compromise” (Panagiotakos personal communication 2019). Alcohol is used as a group, but several different types of alcohol such as vodka and beer are not typical of a Mediterranean type diet. Red wine is often quoted as typical of the Mediterranean diet but Panagiotakos chose not to use individual drinks. The score of 5 for <300 is less than ideal, since small amounts of alcohol get the same score as zero alcohol.

Potatoes are another interesting food category. Potatoes are not normally listed as a constituent of the Mediterranean diet. In this scoring system all potatoes are dealt with equally. It seems odd that large quantities of French Fries could score a “healthy” 5.

Finally, the question arises “should all food types be weighted the same?” It might be argued that they should not, but one of the benefits of Panagiotakos’ scoring system is its ease of use and complex formulae and adjustments would take away this benefit.

Below is a brief description of the components of Panagiotakos et al. (2007) MedDietScore and how they were applied in the current study:

1. Non-refined cereals (whole grain bread and pasta, brown rice, etc.). One serving was defined as one slice of bread or half cup of rice or pasta per day multiplied by 7 to give a weekly amount (Panagiotakos et al. 2007). The FFQ asked for frequency of consumption of "one slice" of bread, a "medium serving" or "one bowel" of rice or pasta so the quantities are assumed to be similar.
2. Potatoes are included in the MedDietScore despite the fact they are not part of the Mediterranean pyramid which forms the basis of the score. They are included because they are a "good source of vitamins C, B1 and B2, niacin, carbohydrates, fiber, potassium and magnesium, which have been associated with cardiovascular disease risk markers in previous studies" (Estruch et al.2013). The FFQ for the current study used a "medium" serving as the quantity guide.
3. Fruits are quantified in the MedDietScore as "1 medium piece of fruit" or "1 small apple (80 grams)," Similar measurements were used in the FFQ in this study and the strict scientific definition of fruit which included tomatoes and cucumber as fruit was used.
4. Vegetables were scored per 100g in the MedDietScore and in the current study a "medium serving" was used. Some individual foods such as capers, mushrooms and garlic were evaluated separately and not included in the diet score used here.
5. Legumes are seeds from plants with seed pods that split in half. Examples include: pulses such as lentils, chickpeas, beans, peas, peanuts and carob. The MedDietScore uses servings per week and the "medium serving" used in the current FFQ was converted into the same "per week" unit. For the current study, peas were categorised as legumes and care was taken not to include a food in more than one category. Tofu from the soy bean and beansprouts from the mung bean were also categorised as legumes.
6. Fish is scored in the MedDietScore by aggregating consumption of all types of fish and all ways of cooking, including frying and baking. A low score is allocated when low quantities of fish are eaten and vice versa.
7. For the purposes of scoring red meat consumption, red meat includes meat from beef, lamb, goat and pork species. Meat products include sausages and processed meat from any species, including poultry.

8. Poultry meat is regarded as healthy source of protein by some nutritionists (Farrell 2013) because of its lower fat content, but in the MedDietScore consumption of poultry is scored lower if frequently eaten and higher if never or infrequently eaten since poultry is only eaten occasionally in the traditional Mediterranean diet pyramid.
9. Full fat dairy products include milk and products of milk such as cream, cheese and full fat yoghurt. Eggs are not dairy products but are often grouped together with dairy products since they are animal bi-products. In this study, eggs and avocado (also high in fat) were not included as full fat dairy products. For the purpose of this study "dairy" and "dairy products" refer to milk and milk products derived from mammals such as cows, goats and sheep.
10. Olive-oil consumption was measured in the number of days per week olive oil was used in cooking and as a dressing. Daily use of olive-oil received a score of 5 and never a score of 0. In this study, those who used an alternative fat such as vegetable oil, butter or animal fat to cook were regarded as not using olive-oil whereas those that reported the use of olive-oil were considered to use it daily. This is a potential source of error, but it would be the same in both cases and controls. Olive-oil is used extensively on salads in the Mediterranean counties but does not feature as a salad dressing in the NTFS questionnaire as a result, olive-oil consumption may be artificially lower than it should be in both cases and controls in this study, although the amount is likely to be equal in both groups.
11. Alcohol was calculated in the MedDietScore based on the volume of alcohol consumed. In the NTFS questionnaire and the FFQ used in the current study, alcohol was measured in Units, not by volume, with 1 Unit equivalent to 125ml of 12% alcohol. For comparison with the MedDietScore, the alcohol scores in this study were converted from Units into ml. such that 10 Units= 1250 ml.

There are certainly limitations in using portion size and controversy still surrounds its use (Shim Oh and Kim 2014). Some researchers suggest between-person variations might be highly explained by the portion size rather than the frequency (Kim and Choi 2002). Unfortunately, there was no pre-testing of how the questionnaire was interpreted in relation to portion sizes. This could have been useful.

4.6.3 *Demographic and Socio-economic Information*

Questions on sex, date of birth, ethnicity, place of birth, current area of residence, highest education level, highest qualification, marital status, accommodation ownership, appeared in section 1 of the questionnaire.

Due to regional differences in terminology some adaptations were made to the questions. For example, "Gymnasium" is a school not found in the UK but is a lower secondary school in Cyprus, and education is compulsory up to this level. The "Apolyterium certificate" does not exist in UK but is a type of school leaving certificate.

The socio-demographic information excluding skin type, smoking, alcohol intake, and general health are shown in the descriptive results. The Chi squared test is appropriate for descriptive results and was used for 127 cases and 718 controls and 119 cases and 119 age-matched controls. For the 127 cases and 718 controls, only age was significantly different between cases and controls. This confirms age as a potential confounder and justifies the age-matching.

In the results section The Total Medscore score is tested with each demographic factor in turn using logistic regression or conditional logistic regression in the age-matched analysis. The OR remains unchanged or changed by 2% or less, suggesting none of the socio-demographic factors were confounders.

4.6.4 *Smoking*

Questions on smoking in this study included current smoking, ever/never smoking, exposed to smoke at home and work, inhalation, age when starting quantity per day types of cigarettes, cigars, shisha, and reasons for quitting. If a participant indicated that they had never smoked all subsequent missing data from that person was assumed to be no or zero. When a participant indicated they had smoked at some time subsequent missing data from that person was left as missing and excluded from the analysis. A "smoking status" variable [never smoked, former smoker, current smoker] was created to test for confounding of this factor.

All daily quantities of number of cigarettes smoked were converted to weekly values. The smoking questions from the questionnaire can be seen in Appendix G.

4.6.5 *Alcohol*

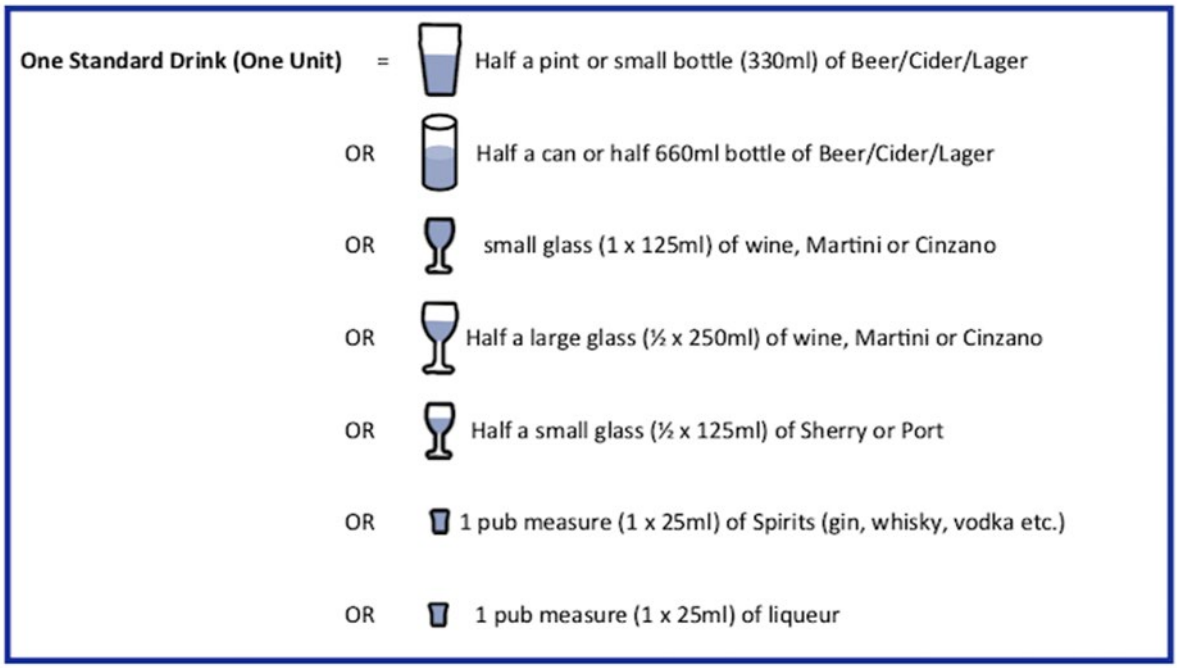
Alcohol consumption was part of the MedDietScore described by Panagiotakos et al. (2007), so these questions were needed in this study.

Questions on alcohol in this study included typical weekly or daily consumption of alcohol, types of drinks consumed including spirits, wine, beer, cider and alcoholic drinks typical of Cyprus such as Zivania. All answers were converted to weekly Units. One unit of alcohol was used as a standard measure in all questions.

The alcohol questions from the questionnaire in English appear in Appendix G.

If a participant indicated they never drank alcohol and left all the other questions in this section blank, it was assumed that all the answers to the blank questions were zero. In order to fill out the alcohol questions Figure 16 was used as guidance.

Figure 15 The Newcastle Thousand Families Study alcohol guidance figure



4.6.6 *General Health*

Height and weight were self-reported in the questionnaire and were used to calculate body mass index (BMI, kg/m^2). BMI categories were classified according to WHO guidelines as follows (Nuttall 2015):

Underweight	BMI < 18.5
Normal (healthy weight)	18.5-25
Overweight	25-30
Obese	>30

4.7 Statistical Analysis

Descriptive statistics were used to describe the basic features of the data in the study to provided simple summaries about the sample and the measures. The four major types of descriptive statistics used are described below. Data were tested for normality by comparing histograms of age. Where the data were not normal, summary data are shown as medians with inter-quartile (IQR) range. Summary data for normally distributed data are shown as means with standard deviation (SD) Statistical analysis was done using STATA 15.1 (StataCorp 15.1 for Mac 2018). A Chi square test was used to compare distribution of estimated intake for cases and controls.

The Logistic regression analysis

Logistic regression analysis is the analysis of choice for case-control studies, and this method was used to compare the risks of various factors for example, diet, with multiple

sclerosis. Odds ratios with confidence intervals (CI) and p values were obtained and then a dose response was calculated for each variable of interest. A dose response is when there is a biological gradient in the association between a variable (for example vegetable intake) and the outcome (for example multiple sclerosis). In other words, in a dose response, as the amount of vegetable consumption increases, does the likelihood of multiple sclerosis change?

4.7.1 *The Age-matched conditional regression analysis*

The *a priori* objective was to age-match controls after cases had been recruited. In this way, the potential confounding of age can be controlled at the design stage which is the most efficient way of dealing with confounding (Sedgwick 2015). However, recruiting cases took longer than expected and time restraints meant that controls needed to be recruited unmatched at the same time as recruitment of cases. As recruitment of cases and controls came to an end, it became apparent that there was a difference in mean age of cases and controls. When comparing the whole dataset, the mean age of the controls (32 years, SD 12.8, *n* =686) was much younger than the cases (46 years, SD 14 .1, *n* = 123). Similarly, the median age of controls (27 years, IQR 23-38) was lower than the median age of cases (46 years, IQR 3 7-57). With this in mind, it was decided to match cases and controls for age using the same year of birth where ever possible. The 119 cases who gave their age were matched with 119 controls using the following method. Random numbers were generated using STATA 15.1 software and each of the controls was allocated a number. The control data were sorted on age, and cases and controls were matched on year of birth. When there were two or more controls with the same year of birth as the case, the control with the highest random number was used. For example, there was 1 case and 27 controls aged 19 years. The control with the highest random number was matched with the case. The majority of cases and controls were matched in this way. When there was a case but no control of the same year of birth, for example, a case aged 69, then an "unused" control born the year before or the year after was used. Similarly, when there were 4 cases aged 65 and only 1 control, the extra 3 controls were recruited from one year before and one year after. If 2 cases had the same age, then 2 controls with the highest random numbers were used. All the cases were matched to a control born the same year or one year before or one year after. The data from 119 cases and 119 controls were analysed using conditional regression analysis. Odds ratios with confidence intervals (CI) and p values were obtained and then a dose response was

calculated for each variable of interest. Unfortunately, there were not enough controls of the same age to allow two controls to be matched with each case.

Defining "dose response"

After assessing if cases and controls were exposed to a variable in a binary manner, for example ate a food or not, a dose response association was calculated. With a "dose response" association, for every step increase in the exposure of interest, there is an accompanied stepwise increase or reduction in the likelihood of the outcome (for example having multiple sclerosis).

4.7.2 *Odds ratios*

The odds ratio provides a measure of the association between the exposure and the outcome. In a case-control study, the odds ratio estimates the odds of exposure among cases compared with controls (Sedgwick 2013). The OR is considered a good estimate of the risk in rare diseases such as multiple sclerosis and was used in the current study. When there is dichotomous outcome e.g. yes/no an OR calculated by logistic regression is the appropriate test to use (Sedgwick 2013). ORs are quoted in case-control studies together with the predetermined level of statistical significance (p value) and the calculated confidence interval quoted. The 95% confidence interval (CI) is a range of values that one can be 95% certain contains the mean.

When there is matching for age (the so-called age-matched analysis) the OR is calculated using conditional logistic regression.

4.7.3 *Ordinal variables*

Ordinal variables are similar to categorical variables except ordering is clear. For example, in this study, BMI was recorded on a continuous scale then, categorised into Underweight, Healthy, Overweight and Obese with the lowest BMI being Underweight and the highest Obese. Therefore, obesity is an ordered categorical (ordinal) variable. As another example, some people ate zero apples per week, others ate 7, 14 or 21 per week. It was possible to convert these ordinal data into nominal data. For example, ate zero apples or ate some apples. In this case the exposure to the variable is described as binary. Odds ratios with confidence intervals (CI) and p values were then calculated.

Chapter 5. Results

The results of the study are presented in this chapter. Initially, the results were analysed with and without matching. Since the results were very similar, only the age-matched results are included in this chapter. The un-matched results are now included in Appendix A.

Section 5.1 includes the descriptive statistics and the results of the demographic characteristics of cases and controls are stated and compared. How the missing data impacts on the demographic results are stated. The significant difference between age of cases and controls is described and the decision to undertake an age-matched conditional regression analysis is reported. The methodology chapter covered the age-matched conditional regression analysis. Section 5.2 deals with the main focus of the thesis. It examines the association between a Mediterranean type diet and multiple sclerosis, using a score developed by Panagiotakos (Panagiotakos et al. 2007). The Mediterranean diet score adjusted for each potential confounder in turn is then presented. Following this, any data on food groups that are part of the Mediterranean diet score are presented. Then, any individual foods that may influence the risk of acquiring multiple sclerosis are presented. Section 5.3 presents the smoking data, and section 5.4 the alcohol data as standard predictors. Section 5.5 presents the impact of the missing data. Section 5.6 deals summerises the hypothesis testing.

5.1 Descriptive results

There were 119 multiple sclerosis cases with a consultant diagnosis who provided their age and who returned the questionnaire in the time period of the study (August 31st 2017 to August 31st 2018). These were matched with 119 controls as described in Chapter 4. A summary of demographic information appears in Table 6.

Table 6 Summary of demographic information used in the age-matched analysis.

	Controls (n=119) n (%)	Cases (n=119) n (%)	Total n (%)	Chi² p-value
Sex				
Men	47 (39.5)	40 (33.6)	87 (36.6)	0.35
Women	72 (60.5)	79 (66.4)	151 (63.5)	
Age	Matched	Matched		
BMI Categories				
Underweight	4 (3.4)	8 (7.1)	12 (5.2)	
Healthy	69 (59.0)	58 (51.3)	127 (55.2)	
Overweight	33 (28.2)	31(27.4)	64 (27.8)	
Obese +severely obese	11 (9.4)	16 (14.2)	27 (11.7)	
Total				
Mean BMI	117	113	230	
	23.39 SD 5.15	24.68 SD 4.82		0.09
Place of birth				
Nicosia	49 (41.2)	49 (51.0)	98 (45.6)	
Limassol	24 (20.2)	16 (16.7)	40 (18.6)	
Larnaca	19 (16.0)	13 (13.5)	32 (14.9)	
Famagusta	9 (7.6)	5 (5.2)	14 (6.5)	
Paphos	2 (1.7)	2 (2.1)	4 (1.9)	
Other eg Greece, UK	16 (13.5)	11 (11.5)	27 (12.6)	
Total	119	96	215	0.80
Place of current residency				
Nicosia	77 (64.7)	60 (57.7)	137 (61.4)	
Limassol	23 (19.3)	23 (22.1)	46 (20.6)	
Larnaca	16 (13.5)	16 (15.4)	32 (14.4)	
Famagusta	2 (1.7)	3 (2.9)	5 (2.2)	
Paphos	1 (0.8)	2 (1.9)	3 (1.4)	
Total	119	104	223	0.80
Ethnicity				
Greek Cypriot Yes	114 (95.8)	111 (96.5)	225 (96.2)	
No	5 (4.2)	4 (3.5)	9 (3.9)	
Total	119	115	234	0.77
Maternal ethnicity				
Greek Cypriot Yes	111 (93.3)	108 (93.1)	219 (93.2)	0.96
No	8 (6.7)	8 (6.9)	16 (6.8)	
Total	119	116	235	
Paternal ethnicity				
Greek Cypriot Yes	114 (95.8)	109 (94.8)	223 (95.3)	
No	5 (4.2)	6 (5.2)	11 (4.7)	
Total	119	115	234	0.71
Housing status				
Owned	88 (75.9)	82 (71.3)	170 (73.6)	
Rented	20 (17.2)	24 (20.9)	44 (19.0)	
Other	8 (6.9)	9 (7.8)	17 (7.4)	
Total	116	115	231	0.75
Marital status				
Cohabiting	7 (5.9)	4 (3.4)	11 (4.3)	
Divorced	5 (4.2)	7 (6.0)	12 (5.1)	
Married	78 (65.5)	80 (69.0)	158 (67.2)	
Single	22 (18.5)	21 (18.1)	43 (18.3)	
Widowed	7 (5.9)	7 (6.0)	11 (4.7)	
Total	119	116	235	0.66
Highest qualification				
No qualification	16 (15.1)	11 (10.3)	27 (12.7)	
Apolyterium/ GCSE	40 (37.7)	46 (43.0)	86 (40.4)	
BA/BSC	18 (17.0)	29 (27.1)	47 (22.1)	

Masters/ PHD	32 (30.2)	21 (19.6)	53 (24.9)	
Total	106	107	213	0.13

There was no significant difference between cases and controls once age had been matched.

Descriptive results for smoking are shown in Table 7.

Table 7 Smoking Status

	Never smoked n (%)	Former smokers n (%)	Current smokers n (%)	Total
Controls	42 (42.9)	15 (12.6)	62 (68.9)	119
Cases	56 (57.1)	35 (29.4)	28 (31.1)	119
Total	98 (41.1)	50 (21.0)	90 (37.8)	238

Pearson chi2(2) = 22.8 p = 0.000

5.2 The Mediterranean diet

Descriptive table and summaries of a Mediterranean diet score adapted from the MedDietScore

A Mediterranean diet score (Medscore) derived from the MedDietScore (Panagiotakos et al, 2007) scoring system was used to assess adherence to the Mediterranean diet as described in the methodology chapter. Each of 11 food categories was given a score. The maximum total Mediterranean diet score was the sum of the 11 components (10x5) +3(olive oil) = 53. The minimum and maximum scores for the cohort were 12 and 50, respectively. Cases had a slightly lower mean score of 33.3 (SD 5.24) and ranged from 17 to 46 while the mean score for the control group was 33.9 (SD 5.74) and ranged from 12 to 50. There was no statistical significance between the mean Medscore of cases and controls (p= 0.26)

Defining "dose response"

After assessing if cases and controls were exposed to a variable in a binary manner, for example ate a food or not, a "dose response" association was calculated. With a "dose response" association, for every step increase in the exposure of interest, there is an

accompanied stepwise increase or reduction in the likelihood of the outcome (for example having multiple sclerosis).

The mean Medscores of the 11 food categories were calculated for the age-matched cohort and are seen in Table 8.

Table 8 Mean Medscores of the 11 food categories in this cohort

	Controls (n=119) Mean (SD)	Cases (n=119) Mean (SD)
Alcohol	3.40 (2.30)	2.44 (2.51)
Red meat and products	2.72 (1.76)	2.82 (1.49)
Poultry	4.77 (0 .76)	4.83 (0 .72)
Fish	2.37 (1.48)	2.54 (1.25)
Non-Refined cereals	2.25 (1.12)	2.00 (1.03)
Potatoes	3.35 (1.32)	2.97 (1.34)
Dairy products	3.74 (1.42)	4.08 (1.33)
Fruit	4.42 (1.00)	4.24 (1.21)
Vegetables	3.11 (1.27)	2.72 (1.26)
Legumes	2.99 (1.16)	2.60 (1.04)
Olive oil	2.21 (1.33)	1.87 (1.46)

The Total Medscore in relation to multiple sclerosis unadjusted and adjusted for sex is shown in Table 9 and shows sex is not a confounder, with no change in the odds ratio.

Table 9 The Total Medscore in relation to multiple sclerosis. Unadjusted, and with adjustments using conditional regression analysis

Age-matched conditional regression analysis	Total Med Diet Score OR [95% CI] p
Unadjusted	0.91 (0.85, 0.96) **0.001**
Adjusted for sex	0.91 (0.85, 0.97) **0.002**

For each unit of the Total Medscore there was a 9% reduction in the likelihood of the outcome (multiple sclerosis).

Potential demographic confounders in relation to the Mediterranean diet score are shown in Table 10 and it can be seen these factors are not confounders, since the odds ratio does not change by more than 10%.

Table 10 The Total Medscore in relation to demographic potential confounding factors adjusted one at a time.

	Total Med Diet Score OR [95% CI] p
Unadjusted	0.91 (0.85, 0.96) **0.001**
Adjusted for sex	0.91 (0.85, 0.97) **0.002**
Adjusted for body mass scale	0.90 (0.83, 0.96) **0.002**
Adjusted for ethnicity	0.91 (0.86, 0.97) **0.004**
Adjusted for mother's ethnicity	0.91 (0.86, 0.97) **0.002**
Adjusted for father's ethnicity	0.91 (0.85, 0.97) **0.002**
Adjusted for place of birth	0.91 (0.85, 0.98) **0.01**
Adjusted for current residency	0.89 (0.83, 0.95) **0.001**
Adjusted for Smoking status	0.90 (0.85, 0.97) **0.002**
Adjusted for Regularly exposed to smoke	0.91 (0.85, 0.96) **0.001**

Smoking status and being "regularly exposed to smoke" were not confounders since the odds ratio does not change by more than 10%.

Alcoholic drinks as potential confounders are shown in relation to The Total Medscore in Table 11.

Table 11 Alcoholic drinks as potential confounders

	Total Med Score
Unadjusted For sex	0.91 (0.85, 0.96) **0.001**
Adjusted for sex	0.91 (0.85, 0.97) **0.002**
Adjusted for and beer, cider per week	0.94 (0.88, 1.01) 0.08
Adjusted for units of spirits per week	0.90 (0.84, 0.96) **0.002**
Adjusted for fortified wines per week	0.91 (0.85, 0.97) **0.003**
Adjusted for and wine per week	0.91 (0.85, 0.97) **0.006**
Adjusted for red wine per week	0.91 (0.85, 0.97) **0.004**
Adjusted for white wine per week	0.91 (0.85, 0.97) **0.005**
Adjusted for rose wine per week	0.91 (0.85, 0.97) **0.003**

The consumption of alcoholic drinks was not found to be a confounding factor.

The individual food group Medscores were analysed and the results are shown in Table12.

Table 12 Individual food group Medscore unadjusted for sex and adjusted for sex

Med score for:	Unadjusted OR [95% C.I. p]	Adjusted for sex OR [95% C.I.] p
Vegetables	0.77 (0.62, 0.96) **0.02**	0.76 (0.61, 0.95) **0.02**
Dairy products	1.21 (0.99, 1.47) 0.06	1.24 (1.01, 1.52) **0.04**
Alcohol	0.83 (0.73, 0.94) **0.002**	0.83 (0.73, 0.94) **0.003**
Legumes	0.73 (0.57, 0.93) **0.01**	0.72 (0.56, 0.92) **0.01**
Potatoes	0.81 (0.66, 0.98) **0.03**	0.81 (0.66, 0.99) **0.04**
Non-refined cereals	0.78 (0.60, 1.02) 0.06	0.79 (0.6, 1.02) 0.07
Olive oil	0.86 (0.71, 1.04) 0.12	0.87 (0.71, 1.05) 0.14
Red meat and products	1.04 (0.89, 1.23) 0.62	1.04 (0.88, 1.22) 0.66
Poultry	1.13 (0.80, 1.61) 0.49	1.17 (0.82, 1.68) 0.39
Fish	1.09 (0.91,1.30) 0.37	1.10 (0.92, 1.32) 0.32
Fruit	0.86 (0.68, 1.09) 0.21	0.85 (0.67, 1.08) 0.18

The Medscore scores for consumption of vegetables, alcohol, legumes and potato were significantly negatively associated with being a multiple sclerosis case and this association followed a dose response. In contrast, the Medscore for dairy products was significantly positively associated with being a multiple sclerosis case when adjusted for sex and this score followed a dose response.

In summary, a statistically significant association existed between certain food groups that contributed to the Mediterranean diet score.

The individual foods were then examined in a binary association (ate this food or not) and for a "dose response" the frequency of consumption was used. A summary of the statistically significant results of individual foods is shown in Table 13.

Table 13 Summary of individual foods with a dose response

	Unadjusted for sex		**Adjusted for sex**	
		Dose response for a 1 unit increase		**Dose response for a 1 unit increase**
Food	**OR (95% CI) P**	**OR (95% CI) P**	**OR (95% CI) p**	**OR (95% CI) p**
Beetroot	0.15 (0.04, 0.68) **0.01**	0.71 (0.55, 0.91) **0.01**	0.16 (0.04, 0.71) **0.02**	0.71 (0.55, 0.91) **0.01**
Grapefruit	0.21 (0.06, 0.75) **0.02**	0.79 (0.66, 0.94) **0.01**	0.20 (0.06, 0.71) **0.01**	0.79 (0.66, 0.95) **0.01**
Dried pulses	0.22 (0.08, 0.66) **0.01**	0.83 (0.70, 0.98) **0.03**	0.22 (0.08, 0.66) **0.01**	0.83 (0.70, 0.98) **0.03**
Oat based cereal	0.14 (0.03, 0.63) **0.01**	0.90 (0.81, 1.00) **0.04**	0.13 (0.03, 0.60) **0.01**	0.90 (0.82, 1.00) **0.05**
Refined breakfast cereal	0.15 (0.05, 0.51) **0.002**	0.88 (0.78, 0.99) **0.03**	0.15 (0.04, 0.49) **0.002**	0.88 (0.78, 0.99) **0.04**
Tomatoes	0.25 (0.05, 1.18) 0.08	0.95 (0.91, 1.00) **0.05**	0.25 (0.05, 1.17) 0.08	0.95 (0.91, 1.00) **0.05**
Whole Meal pasta	0.36 (0.13, 0.99) **0.05**	0.79 (0.63, 1.00) **0.05**	0.37 (0.13, 1.03) 0.06	0.79 (0.62, 1.00) **0.05**
Tofu	0.38 (0.15, 0.96) **0.04**	0.37 (0.13, 1.03) 0.06	0.38 (0.15, 0.98) **0.05**	0.37 (0.13, 1.05) 0.06

As can be seen in Table 13, certain individual foods were significantly negatively associated with being a multiple sclerosis case. This association was found to follow a dose response with some foods. That is, a linear inverse association exists between the consumption of these variables and multiple sclerosis.

Consumption of tofu was significantly negatively associated but did not follow a dose response.

The individual foods for the un-matched data are shown in Appendix B when examined in a binary association and for a dose response.

Analysis of foods avoided.

A summary of the results of the foods avoided by cases is shown in Table 15. There were 34 cases who reported avoiding some foods, and one person avoided all “take-aways”. Only 3 cases gave a reason for avoiding food, and when available, the reasons given are included.

Table 14 Results of foods reported to be avoided by cases

Food	Cases	Total foods avoided	Reason
Avocado	1	1	Fat
Beef/red meat/meat	6	10	-
Broad beans	1	1	Allergy
Beans	1	3	-
Black eyed peas/beans	2	2	-
Butter beans	1	1	Indigestion
Eggs	1	1	-
Fried food	7	10	-
Dairy products	3	6	-
Fat	2	3	-
Fish	1	1	-
Mushrooms	1	1	-
Onions	1	1	-
Garlic	1	2	-
Pulses	1	1	-
Shellfish	1	1	-
Sugar	1	1	-
All vegetables	1	1	-
Wheat	1	1	-
Total	**34**	**48**	

One person avoided dairy and fatty food, one person avoided red meat and dairy. Another person avoided all fried food and beans. One person avoided red meat and garlic. One person avoided pork and all fried food. Another, all fried food and all meat. One person

avoided all dairy food, salt and fats. The most commonly avoided foods were beef and red meat and fried food with 6 and 7 cases, respectively, reporting that they avoided these food types. Other foods avoided were various types of vegetables, dairy and fish/shellfish.

Analysis of foods eaten by cases to help their medical condition

The results of foods eaten to help their medical condition is shown in Table 15. Only 32 cases responded and there were no reasons given for this action, or whether it changed since diagnosis.

Table 15 Foods to help with the medical condition

Food	Cases
Bananas, nuts, oats	1
Chicken, Pulses	1
Chicken, Salad, Potatoes	1
Fish	5
Fish, Nuts, Eggs, Veg	1
Fruit, Fish, Veg	2
Grilled food and salad	1
Meat	1
Pasta, Rice, White	1
Salad and Pulses	1
Salad, Chicken	1
Veg, beans, salad	1
Fish, chicken, nut	1
Fruits, vegetables	10
Greens vegetables, fish	1
Salads, salmon	1
Salmon	1
Whole grain, chicken	1
Total	**32**

5.3 Analysis of smoking data

Descriptive smoking results appear in section 5.1 and the results of smoking and exposure to smoke as potential confounders is reported in the results of the Mediterranean diet scores in section 5.2 In this section, smoking status and being “regularly exposed to

smoke" are shown not to be confounders since the odds ratio does not change by more than 10%.

5.4 Analysis of alcohol consumption data

The type of alcohol consumed per week is shown in Table 16. The binary data refers to whether a case drinks that type of alcohol or not when compared to a control.

Table 16 Type of alcohol consumed per week

	Binary age-matched Unadjusted for sex OR [95% CI] P	**Binary age-matched and adjusted for sex OR [95% CI] P**
Units spirits/week	0.67, (0.35, 1.26) 0.21	0.71(0.37, 1.37) 0.31
Fortified wines/week	1.05e-17 (0.00) 1.00	4.92e-08 (0.00) 0.99
Beer /cider/week	0.24 (0.13, 0.47) **< 0.001**	0.24 (0.12, 0.47) **< 0.001**
White Wine	0.61 (0.35, 1.09) 0.09	0.63 (0.35, 1.12) 0.12
Rose Wine	0.29 (0.11, 0.80) **0.02**	0.31 (0.11, 0.83) **0.02**
Red Wine	0.52 (0.28, 0.94) **0.03**	0.53 (0.28, 1.00) **0.05**
Wine/week	0.61 (0.35, 1.09) 0.09	0.63 (0.35, 1.12) 0.12

There were statistically significant associations between being a case and drinking beer /cider, rose wine and red wine. However, spirits and white wine were not statistically significant either unadjusted or adjusted for sex. Cases were 76% less likely to drink beer and cider, 69% less likely to drink rose wine and 47% less likely to drink red wine. Too few people drank fortified wine to provide reliable odds ratios and confidence intervals. The results of each alcoholic drink as a potential confounding factor have been described in relation to The Medscore in section 5.2. None of the alcohol drinks were considered to be confounding factors.

Cases vs controls

The difference in missing data in cases and controls varied depending on the question. It was beyond the scope of this thesis to analyse every question in the questionnaire on the basis of available or missing data. However, some general remarks can be made using the demographics section as an example. Available data for some questions were generally high in both cases and controls. For example, sex was known for 100% of cases and controls. In the age matched analysis, controls were selected randomly and matched to cases on age, so the amount of missing data of cases and controls was unknown until analysis. There was no attempt to choose controls with more data.

Smoking questions

All of the participants responded to the question if they currently smoke or not or ever smoked or not or were regularly exposed to smoking or not. However, when a respondent said they currently did not smoke and had never smoked, it can be assumed they did not smoke manufactured cigarettes, rolled cigarettes and any type of cigars. Some of these respondents had left the section on ways of smoking blank. It has been assumed that these responses were null rather than blank. All other blanks were recognised and coded as blank responses. As a result, the smoking data are complete in some areas and incomplete in others. When quantities were required, or free text spaces for answers were available, many more blanks appeared. When the answer was yes or no, respondents were more likely to provide an answer. Some questions had considerable amounts of missing data. Where no assumptions could be made, gaps were entered as blanks. For example, “filters used”. About 1/3 of these data were missing.

Alcohol

When asked how much alcohol they consumed per week, many respondents reported zero and some left this and other questions blank. For all other questions on wine, beer or spirit consumption those people who reported zero initially and left the other questions blank were assumed to drink zero units of all types of alcohol per week.

A Mediterranean diet score and individual foods

A Mediterranean diet score was calculated for all participants. Missing data on individual foods was generally low, so it was still possible to calculate a value for each of the categories in the Medscore system.

Free text questions

Free text questions generally had few responses.

5.6 Hypothesis testing

The *Null hypothesis* tested was that there is no difference in Mediterranean diet pattern score, or intake of specific food groups, or foods, between multiple sclerosis cases and control participants.

The results presented in this chapter indicate that the *Null hypothesis* can be rejected, and the alternative hypothesis accepted. That is, there are differences in the Mediterranean diet pattern score, or intake of specific food groups, or foods, between multiple sclerosis cases and control subjects.

Chapter 6 Discussion and Conclusion

The discussion of the results of the study are presented in this chapter. The discussion section will relate this study to previous research and highlight the importance of this study to others. The implication of these results to clinical practice and preventative medicine, where appropriate, will be highlighted. Strengths and limitations will be examined, and suggestions for future research will be made.

6.1 Summary of the main findings

In this case-control study of 718 controls and 127 cases, patients with multiple sclerosis when compared with controls, had a lower Mediterranean diet score (MDS). These results appear in Appendix A. MDS dose response adjusted for age OR 0.96 [95% CI] (0.93, 1.00) p 0.04

Similarly, in the aged-matched case-control study of 119 controls and 119 cases, patients with multiple sclerosis when compared with controls, had a lower Mediterranean diet score. MDS dose response OR 0.91 [95% CI] (0.85, 0.96) p 0.001. After statistical adjustment for each potential confounder in turn, the results of the Mediterranean diet score in both analyses remained unchanged and were statistically significant. Cases were compared to controls in their consumption of food groups. Medscore scores for consumption of vegetables, alcohol, legumes and potato were significantly negatively associated with being a multiple sclerosis case and this association was found to follow a

dose response for vegetables, alcohol and legumes. Finally, cases were compared to controls in their consumption of individual foods. Grapefruit, tomatoes, onions, refined breakfast cereal, dried pulses and beetroot consumption were significantly negatively associated with being a multiple sclerosis case. This association was found to follow a dose response. That is, a linear inverse association existed between the frequency of consumption of these foods and the presence of multiple sclerosis.

One interpretation of this finding is that lower adherence to the Mediterranean diet (as indicated by the lower Medscore) has increased the risk of acquiring the disease. This idea makes several assumptions. Firstly, that the diet of the multiple sclerosis cases, while different now from the controls, was also different in the past before disease diagnosis. There is no evidence to support or contradict this. However, the fact that multiple sclerosis cases did not indicate a diet change in answers in the questionnaire tends to suggest that their diet was likely to be similar if not the same as it was before diagnosis, although we cannot be certain of this, as there is no evidence one way or the other. Unfortunately, controls were not asked about historical dietary patterns.

Adult dietary patterns may change over time. Mishra et al. (2006) suggest they do. They conducted a longitudinal cohort study of 1265 participants and assessed their diet in 1982, 1989 and 1999. They found marked changes over time. In this study, it was not possible to assess if diet changes had occurred in either cases or controls over time.

The second assumption is that diet can directly influence the risk of developing multiple sclerosis. At the outset, this study tried to explore diet and the risk of developing multiple sclerosis, and while statistical evidence supports an association, a study of this type can provide no more than an association. While diet is well documented as a risk factor in cardiovascular disease, this has not been established yet in multiple sclerosis. The lower adherence to the Mediterranean diet pattern was shown in the diet of cases who were less likely to eat some foods and food groups that contribute to the Mediterranean diet. These findings remained significant after adjusting for potential confounders such as age and sex in the logistic regression analysis, and sex in the age-matched analysis, suggesting that diet may be a significant factor in the aetiology of multiple sclerosis development. The significant relationship between the Mediterranean diet, certain food groups and individual foods may be important in development of the disease in a susceptible individual.

Interpretation of the study findings in the context of the existing evidence in this area and suggestions for future work.

The findings of this study are in broad agreement with those of the only other case-control study. They are also in broad agreement with those studies of the Mediterranean diet and cardiovascular disease and colorectal cancer. As stated earlier, diet has not been established yet as a risk factor for multiple sclerosis. This study adds to the current knowledge on the topic.
Further work might include large cohort studies and dietary intervention studies. Future work is discussed later in the chapter.

6.2 The Mediterranean diet

Low grade inflammation has been extensively studied for its association with cardiovascular disease, which may have a similar pathophysiology to multiple sclerosis, as both involve chronic inflammation. Biomarkers such as C-reactive protein, white blood cell counts and circulating fibrinogen have been found to be reliable measures of inflammation (Bonaccio et al. 2017). So-called "healthy diets" such as the Mediterranean diet are regarded as being associated with lower concentrations of markers of inflammation, whereas Western-type diets are often positively associated with inflammation markers (Bonaccio et al 2013). The Mediterranean diet is high in vegetables, whole grains, fruit and nuts, which have been associated with less oxidative stress and lower inflammation. Research has highlighted compounds in these foods that might theoretically provide an antioxidant effect if used as supplements. As yet, clinical trials of dietary supplements are inconclusive, and supplements are not routinely recommended as a measure for preventing cardiovascular disease (Whalen et al. 2016). While there are many studies suggesting an inverse association of cardiovascular disease and the Mediterranean diet there are very few studies which have examined possible relationships between diet and multiple sclerosis. The evidence provided by this case-control study is that there is a possible association between multiple sclerosis and components of the Mediterranean diet. Exposure to the Mediterranean diet was measured on a continuous scale using the Mediterranean diet score (Medscore) and a dose-response calculated. The results show that for one unit increase of the Medscore, there was a 4% reduction in being a multiple sclerosis case. These results are in keeping with the inverse relationship between the Mediterranean diet and multiple sclerosis found by Sedaghat et al (2016). This implies that individuals with multiple sclerosis are less likely to closely follow a Mediterranean type diet than those without the disease, and this is in a step wise manner. The possible reasons are discussed below.

6.2.1 Age-matching

The median age of cases (46 years) and controls (27 years) were different, with a statistically significant Chi^2 p-value. As a consequence, age was judged to be a potential confounder. Age-matching (as described in the methods section) eliminated this potential confounding, and the results were referred to as "the age-matched analysis".

In the age-matched analysis, the Mediterranean diet score was lower in cases than in control participants, with a significant inverse linear association. A one unit increase in the Medscore was associated with a 9% reduction in the outcome (being a multiple sclerosis case).

6.2.2 Comparison to other studies involving multiple sclerosis

In both analyses, there were statistically significant results between cases and controls with regard to the Mediterranean diet scores. This was also the case when adjustments were made for potential confounders such as demographic factors and smoking. Certain food groups and individual foods (as seen in the summary tables) also showed significant associations. These results are in agreement with the observations of Sedaghat et al (2016), who also conducted a case-control study in which they compared the Mediterranean diet score of cases and healthy controls and found that those with multiple sclerosis were less likely to follow the Mediterranean diet. Previously, Ghadirian et al. (1998) had concluded there was a protective role of fruit, vegetables and grain in the risk of acquiring multiple sclerosis and an increased risk with consumption of animal products, a dietary pattern which is similar to the Mediterranean diet. The current study is in broad agreement with these results. For example, when adjusted for age, the following food group scores; vegetables, legumes and non-refined cereals along with alcohol were significantly lower in cases when compared to controls, and dairy products significantly higher in cases. In the age-matched analysis, vegetables, alcohol, legumes and potatoes were significantly lower in cases when compared to controls. Consumption of fish and meats were significantly positively associated with multiple sclerosis, but there were no dose response associations

In the logistic regression analysis, individual foods such as grapefruit, tomatoes, onions, refined breakfast cereal, dried pulses and beetroot consumption were significantly negatively associated with being a multiple sclerosis case.

In the conditional logistic regression analysis, beetroot, grapefruit, dried pulses, oat-based cereal, refined breakfast cereal, tomatoes, whole meal pasta and tofu consumption were significantly negatively associated with being a multiple sclerosis case. This association was found to follow a dose response for all of the above, except for tofu. That is, a linear inverse association exists between the consumption of these variables and multiple sclerosis.

Reasons for different dietary habits of multiple sclerosis patients

There is no clear explanation why cases are less likely to follow the Mediterranean diet compared with control participants. Indeed, the opposite might be expected. For example, Sumowski McDonnell and Bourdette (2017) have suggested that people with multiple sclerosis are usually proactive in seeking a healthier diets and lifestyle in order to delay disease progression and improve symptoms. So, it might have been expected that cases would have a better adherence to the Mediterranean diet than controls, not the other way around.

There are no medical reasons or pharmacological interactions to explain why multiple sclerosis cases would be advised against eating the Mediterranean diet. Instead, there is a great deal of information in the press to suggest to a patient that the Mediterranean diet is a healthy lifestyle option (Fitzgerald et al. 2018), and it might be assumed that on diagnosis, multiple sclerosis cases might switch to this healthy option or even be advised to adopt a healthy diet option. The results of this study suggest that this does not appear to be the case.

6.2.3 *Comparison with other studies involving other diseases*

Colon cancer

The results of previous multiple sclerosis studies and the current study are in broad agreement with studies on colon cancer. Adherence to the Mediterranean diet has been studied extensively with regard to colon cancer. Schwingshackl et al. (2017) undertook a systematic review and meta-analysis of 11 observational studies including 5 case-control studies on adherence to the Mediterranean diet and the risk of colorectal cancer. They pooled the odds ratios and risk ratios (RR) and concluded that with a higher adherence to the Mediterranean diet there was an inverse risk of colorectal cancer (RR 0.82, 95% CI 0.75 to 0.88). For the 5 case-control studies there was a similar reduction in pooled odds

ratio (OR, 0.71, 95% CI 0.57,0.88). These results suggest that colon cancer patients were 29% less likely to consume a Mediterranean diet compared with controls. This is in keeping, although larger in scale, to the age-matched conditional regression analysis results in the current study where cases were 9% less likely to consume a Mediterranean diet compared with controls. The carcinogenesis of colorectal cancer is complex, but a simplistic explanation is that it involves chronic inflammation as seen in Crohn's disease and ulcerative colitis. These two diseases are known to increase the risk of colorectal cancer. It is possible that the protective effect of the Mediterranean diet on colorectal cancer risk is associated with either the sum of the anti-inflammatory effects of the individual foods of the Mediterranean diet or the synergistic effects of the individual foods of the Mediterranean diet (Donovan et al. 2017). The gut microbiota is likely to play an important role in colorectal cancer. For example, the fermentation of dietary fibre resulting in the production of the short chain fatty acid butyrate appears be protective and may prevent tumour formation (Fung et al. 2012). Similar protective effects of the diet might be involved in the risk of acquiring multiple sclerosis. The elements of the Mediterranean diet with the highest anti- inflammatory effects include polyphenolic compounds and unsaturated fatty acids plant-based foods, olive oil and fish oil (Schwingshackl et al. 2017). These are discussed in more detail with reference to multiple sclerosis in a later section.

Cardiovascular disease

There are a number of studies that suggest there is robust evidence that adherence to the Mediterranean diet is protective against cardiovascular events (WHO 1990; Keys 1995; Dinu et al. 2017; Asamudo and Okolo 2019). Sofi (2008) in a meta-analysis of prospective cohort studies states that mortality from cardiovascular disease is 9% lower in those at-risk patients who adhere to the Mediterranean diet. It is possible that the anti-inflammatory effects and antioxidants of the constituents of the Mediterranean diet synergistically reduce chronic inflammation and oxidative stress (Varadharaj et al. 2017). Phenolic compounds found in extra virgin olive oil show antioxidant, anti-inflammatory and antimicrobial properties (Cicerale Lucas and Keast 2012), although the bioavailability of many phenolic compounds is poor, as discussed in a later section. Atherosclerosis involves thickening of the walls of blood vessels in particular, the intima-media. Foods such as fruits, which have high carotenoid and flavonoid components have been shown to be inversely associated with intima-media thickness (Mursu et al. 2007).

Lv et al. (2015) suggest citrus fruits contain secondary metabolites such as flavonoids, alkaloids, coumarins, limonoids, carotenoids and phenol acids. These may be cardiovascular protective and neuroprotective because of their anti-oxidative and anti-inflammatory effects. The pathogenesis of multiple sclerosis is thought to involve inflammatory cytokines, tumour necrosis factor-alpha, interleukin-1β and interleukin-6 leading to demyelination (Heiss et al. 2001). Omega 3 fatty acids found in the Mediterranean diet are said to inhibit tumour necrosis factor alpha, and interferon-gamma. There is therefore the potential of the Mediterranean diet to suppress demyelination of central nervous system nerve cells which occurs in multiple sclerosis (Jahromi et al. 2012). The Mediterranean diet is also high in dietary fibre (Trichopoulou et al. 1995). Fruit, vegetables, legumes, unrefined cereals and nuts all provide a good source of fibre to the diet (Maiani et al. 2009). It has been suggested that dietary fibre reduces the of risks of chronic diseases (Trinidad et al. 2010). At least two separate mechanisms might be taking place. In cardiovascular disease, fermentable dietary fibre is thought to inhibit the enzyme hydroxy-3-methylglutaryl-CoA reductase which is involved in LDL-cholesterol production through the production of propionate (Trinidad et al. 2010). Another possible mechanism is that dietary fibre is thought to alter the gut biome with broader metabolic consequences (Makki et al. 2018). Indeed, the gut biome has been at the centre of considerable research in recent years in relation to neuroinflammation (Feck et al. 2017). Riccio and Rossano (2018) suggest that when investigating the aetiology of multiple sclerosis the gut microbiome is an important consideration. They highlight two particular groups of gut microorganisms. In the first group certain microorganisms flourish in a low fibre (Western-style) diet. While in the second group, (a more diverse group), the gut microorganisms thrive on a vegetable-based diet. These microorganisms are capable of digesting complex oligosaccharides whereas the first group are not (Riccio and Rossano 2018). The authors go on to suggest that the people who eat a Western diet display a reduced number and diversity of the microorganisms that flourish on the vegetable-based diet. They reviewed the gut microorganisms in the vegetable-based diet which appear to have protective and anti-inflammatory properties. They state amongst other benefits this group of organisms reduce oxidative stress and inhibit nuclear factor kappa B which is a proinflammatory transcription factor. It is possible that the cases in the current study had different gut microorganisms when compared with control subjects based on their diet, although it was not possible to test this as faecal samples were not taken for comparison. Oxidative stress is thought to

increase the permeability of the blood brain barrier and be in part responsible for the pathophysiology of multiple sclerosis (Qureshi et al. 2018). Nuclear factor kappa B is a protein that amongst other things, controls cytokine production. If there is irregular control of nuclear factor kappa B, proinflammatory signaling may occur and acute inflammation becomes chronic. As a result, autoimmune diseases like multiple sclerosis can develop (Lawrence 2009).
It seems plausible that the anti-inflammatory activity of the Mediterranean diet could have a protective or ameliorating effect on the inflammatory component of the pathophysiology of multiple sclerosis.

6.2.4 The bioavailability of antioxidants

Plant based foods, olive oil and fish oil are high in phenolic compounds and while polyphenols may have theoretical protective antioxidant properties, the bioavailability of the 8000+ compounds identified as being present in the diet may well be variable. For example, flavonoids have generally low bioavailability (Kawabata Yoshioka and Terao 2019). Despite the generally low bioavailability of phenolics, Pounis et al. (2016), using data from a large population study, concluded the polyphenol content of diet was negatively associated with low-grade inflammation biomarkers. In addition to antioxidant properties, hydroxytyrosol and tyrosol found in extra virgin olive oil may modulate intracellular signalling, scavenge free radicals and down-regulate inflammatory mediators. The gut microbiome also seems to play an important role in affecting the bioavailability of phenolic compounds. For example, Karković Marković et al. (2019) suggest a complex two-way interaction occurs. The gut microbiota enables biotransformation of non-digested phenolic compounds into their catabolic metabolites which increases bioavailability. In addition, phenolic compounds may themselves modulate the composition of the biome.

6.2.5 Food groups of the Mediterranean diet

Vegetables

When compared with controls, cases were calculated to eat less vegetables in both analyses and for one unit increase of the vegetable Mediterranean diet score in the age adjusted analysis there was a 17% reduction in the outcome (being a multiple sclerosis case). A one unit increase in the vegetable score for a person eating no vegetables per week would be simply to eat one serving per week. Or a person eating 1-6 portions a week would need to eat 7-12 portions to increase their score by 1 unit. (see Panagiotakos' MedDietScore system).

In the age-matched analysis the unit change was even higher at around 23% reduction in the outcome (being a multiple sclerosis case) for one unit increase of the vegetable Mediterranean diet score. There would appear no reason why multiple sclerosis patients would deliberately (for health reasons) eat less vegetables than controls. However, it is possible that some multiple sclerosis patients may have a physical disability associated with the disease. Peeling and preparing vegetables, could make this food preparation harder for patients, so this might prevent them from eating as many vegetables, although there was no evidence collected in the study to support this idea. Vegetables contain a wide variety of minerals, vitamins and fibre. Phytochemicals found in some vegetables also have apparent antioxidant properties which are thought to protect against free radical damage (Dias 2012). In some chronic diseases such as cardiovascular disease, free radicals have been implicated in the disease pathophysiology (Zhang et al. 2015). A diet high in vegetables has been shown to be associated with lower risk of cardiovascular disease (Mullie and Clarys 2011) due to possible antioxidant effects and it is possible that such phytochemicals could be protective against multiple sclerosis.

Dairy products

Dairy products are a key source of calcium for example, as well as fat soluble vitamins such as Vitamin D, but are not typical of the Mediterranean diet. In this study cases were found to consume more dairy products than controls, and in a dose dependent manner in both analyses undertaken. It is possible that cases chose to consume more dairy products, perhaps because they erroneously consider dairy products healthy or beneficial for their disease. For example, TV and magazine adverts deliberately portray cows, butter and milk in an idyllic countryside setting or backdrop (Borkfelt et al. 2015).

Alcohol

In both analyses, the Mediterranean diet score for alcohol consumption was significantly lower in cases than controls. For every unit of the Medscore for alcohol, cases were 13% less likely to consume alcohol than controls. There are many studies that suggest alcohol is detrimental to acquiring multiple sclerosis (Pekmezovic et al. 2006). However, there have been other studies that suggest alcohol is potentially protective (Hedström Hillert Olsson and Alfredsson 2014) although others have found no correlation between alcohol consumption and acquiring multiple sclerosis (Massa O'Reilly Munger and Ascherio 2013).

The current study suggests a protective role of alcohol using a Mediterranean diet score for alcohol. In other studies, it has been suggested that alcohol consumption and health follow a J-shaped curve. For example, low levels of alcohol consumption may be beneficial to health, whereas, when the intake level increases to a certain point, excess alcohol consumption becomes harmful (Lungaard et al. 2018). Ruitenberg et al. (2002) suggested one to three drinks per day significantly lowered the risk of dementia (hazard ratio 0·58 [95% CI 0·38–0·90]), giving support to the theory that low levels of alcohol consumption may be neuroprotective.

It is possible that alcohol consumption and acquiring multiple sclerosis also follows a similar J-shaped curve. A j-shaped association was not tested in the current study, but the study found a linear relationship. Andersen et al. (2018) found an inverse relationship between alcohol consumption in adolescence and acquiring multiple sclerosis which was statistically significant in both men and women. For example, women with low alcohol consumption had an odds ratio of 0.56 (95% confidence interval 0.47–0.66) compared with non-drinking women and women with moderate alcohol consumption (OR = 0.49, 95% CI: 0.38–0.62) and high consumption (OR = 0.57, 95% CI: 0.38–0.84).

It is unknown why multiple sclerosis patients in the current study drink less alcohol than controls. The question on how much alcohol is consumed now compared with earlier life showed less consumption in both analyses for cases but the same in both analyses for controls, suggesting cases may have changed their alcohol habit over time.

It is possible that their drinking reflected the adverse media publicity towards alcohol consumption in general in relation to health outcomes. Alternatively, they may have been advised by their doctor or dietician in regard to obesity and other health outcomes. However, there is no evidence to support either suggestion from the present study.

Alcohol in other diseases

In studies of cardiovascular disease, (which may have a similar inflammatory origin to multiple sclerosis), Chiva-Blanch et al. (2011) found that red wine was protective on lipid profile. Estruch et al. (2004) suggest red wine decreased hs-CRP which is a marker for inflammation. It is possible that red wine, which is typical of the Mediterranean diet, is neuroprotective.

Medscore for legumes

In both analyses, cases ate less legumes than control subjects (by 22% in the logistic regression analysis and by 29% in the age-matched analysis). Of the 35 cases who stated they avoided certain foods 6 subjects avoided legumes completely. One person cited an allergy to broad beans while another suggested they got indigestion from butter beans, but no reason was given by the other 4 cases. Martínez-González et al. (2011) state that consumption of legumes generally has declined in the Mediterranean region and they suggest this may be due to an increase in Westernization of the diet and an increase in consumption of the "western diet" in the area (Zhu et al. 2012).

Legumes are a good source of dietary fibre (Trinidad et al. 2010) which has been associated with a healthy lifestyle and to be protective against certain diseases. For example, Fung et al. (2012) suggest that fermentation of dietary fibre is protective against tumour formation in the colon due to the effects of butyrate produced during fermentation of the fibre. Legumes are said to have a positive effect on control of type 2 diabetes reducing HbA1C and blood glucose concentrations (Jenkins et al. 2012). Legume consumption has been associated with hypertension management and a systematic review by Jayalath et al. (2013) concluded that pulses significantly lowered blood pressure in people with and without hypertension. It is not certain that multiple sclerosis has similar pathophysiology to any of the diseases mentioned but it is plausible that any anti-inflammatory effects of the Mediterranean diet would be beneficial in multiple sclerosis disease prevention.

Medscore for non-refined cereals (whole grain food)

In the logistic regression analysis cases are 19% less likely to consume non-refined cereals compared with controls. In the age-matched analysis, cases were 22% less likely to consume non-refined cereals such as whole grain food compared with controls, but this difference was not statistically significant. There is no obvious explanation to explain why cases ate this way. These results are similar to those found using different

methodology by Jahromi et al. (2012). In a cross-sectional Iranian study, a factor analysis was used to identify 7 dietary patterns. In a diet high in whole grains an inverse relationship was noted with multiple sclerosis, and the prevalence of multiple sclerosis was higher in those who had a diet low in whole grains. Sedaghat et al. (2016) found a positive association between refined grains consumption and increased risk of multiple sclerosis.

Medscore for red meat and products

In the logistic regression analysis, cases were 1.18 times more likely than controls to eat red meat and products when the data were unadjusted for age but when adjusted for age the statistical significance was lost and there was no statistically significant dose response.

In the age-matched analysis there were no statistically significant results for cases eating red meat and products. Baghera et al. (2014) also found no significant difference between 113 cases and 113 controls with regard to consumption of red meat. In another study Black et al. (2019) concluded there was inconclusive evidence associated with red meat consumption and the risk of multiple sclerosis. These observations are stark contrast to the association of red meat and cardiovascular disease (Wang et al. 2018). It is possible the consumption of red meat and products are pro-inflammatory, and this may account for the observations in relation to cardiovascular disease but why the data are inconclusive with regard to multiple sclerosis is unknown.

Potatoes

In the logistic regression analysis, there were no statistically significant results for cases eating less potatoes than controls. However, in the age-matched analysis, cases were about 19% less likely to eat potatoes compared with control subjects. It has not been possible to provide a logical explanation to this result.

6.2.6 *Individual Foods*

Individual food items avoided by multiple sclerosis patients

Only 35 cases reported avoiding certain foods as can be seen in Chapter 5 Table 23. Of these, red meat, dairy produce, eggs, fried food and fat accounted for more than half of the foods avoided. The reasons for food avoidance were usually not given, but it is

possible that they had been advised against eating these "unhealthy foods" by their medical team as it is well known these products can influence inflammation. However, as stated earlier there is no evidence to suggest these foods influence multiple sclerosis risk.

Individual food items eaten by multiple sclerosis patients to help their condition

There were 34 cases who reported eating certain foods to help manage their condition. There was often more than one food group listed by a person as shown in the results section of Chapter 5 Table 26. Around a third of those patients who responded, deliberately ate more fish and a third ate more fruit. While two thirds ate more vegetables because of their disease. One might assume the deliberate fish consumption was to raise omega-3 while the deliberate consumption of fruit and vegetables might have been because these foods are regarded by the medical team as "healthy". There are many reports in the press that a "healthy diet" results in less relapses, so it is reasonable to assume this could have motivated some multiple sclerosis patients to adopt this eating pattern. It is of interest to understand why more of the patients did not adopt a "more healthy lifestyle." Despite the deliberate attempt to eat fruit and vegetables by some individuals, overall, multiple sclerosis cases were still less adherent to a Mediterranean diet pattern than controls.

Grapefruit

In both analyses, cases reported eating less grapefruit than control subjects and this appears to follow a dose response relationship. There are several possible explanations. Grapefruit is known to interact with at least 85 drugs (Bailey et al. 2013) and patients taking these drugs are advised to avoid consuming this fruit. There are no documented multiple sclerosis medications that interact with grapefruit (personal communication 2019). However, multiple sclerosis patients who suffer from trigeminal neuralgia might be prescribed carbamazepine which is an anti-epileptic drug with success in treating nerve pain (Patsalos 2016). Grapefruit is known to interact with carbamazepine increasing the level of the drug, so multiple sclerosis patients taking this drug might have been warned against eating grapefruit.

It is possible that multiple sclerosis patients are on medication in addition to their multiple sclerosis medication that interact with grapefruit such as some statins and have been advised not to eat it or drink grapefruit juice.

The median age of multiple sclerosis patients in this study was about 14 years higher than the controls. Older people are more likely to take medication for conditions that are affected by grapefruit than younger patients. An example would be older patients are more likely to need to take statins for hypercholesterolaemia. This study has adjusted for the age effect, and the age-matched analysis showed the same result, so age cannot, on its own, explain the difference between cases and controls. Another possibility is that grapefruit is neuroprotective. In addition to carbohydrates, proteins, vitamins and minerals, grapefruit contains phytochemicals such as flavonoids. These are not essential dietary elements but have been shown to be anti-inflammatory (Lv et al. 2015). Neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease are thought to be driven by a number of factors including oxidative stress (Enogieru et al. 2018). The antioxidant effect of rutin has been proposed as potentially neuroprotective (Budzynska et al.2019), but rutin has poor bioavailability (Faggian et al. 2016) due to a combination of poor absorption, high metabolism and rapid excretion (Enogieru et al. 2018). The literature search suggests that the effect of rutin has not been studied in multiple sclerosis despite the pathogenesis in multiple sclerosis being partly due to oxidative stress (Gilgun-Sherki Melamed and Offen 2004). Rutin is a glycoside of the flavonoid quercetin and is found in many fruits including grapefruit (Enogieru et al. 2018). If grapefruit is indeed protective, the role of rutin should not be ignored and warrants further investigation.

Tomatoes

Compared with controls, multiple sclerosis cases ate significantly less tomatoes and in a dose response manner. The answers from the “foods avoided” question gave no indication that multiple sclerosis patients deliberately avoided eating tomatoes and there are no plausible reasons why this might have occurred.

Tomatoes contain vitamins A, C and K which may hold health benefits for people with chronic inflammation. They also contain copper, potassium, manganese and molybdenum. They are high in dietary fibre and biotin (vitamin B7). They contain all the major carotenoids, namely alpha- and beta-carotene, lutein and lycopene. In particular, quantities of lycopene are high. This carotenoid, which is also found in pink grapefruits and watermelons (Dasgupta and Klein 2014), has been shown to be a strong antioxidant (Riccio and Rossano 2015) and have anti-inflammatory properties (Chen Huang and Chen 2019). Lycopene can also be found in all tomato products such as sun-dried tomatoes, tomato juice, tomato paste, and ketchup and the processing both concentrates

and increases the bioavailability of lycopene (Başaran Bacanlı and Başaran 2017). Lycopene levels are higher in the skin than the pulp of the fruit. It is a lipid soluble chemical, so when consuming tomatoes or their products with oil, there is better absorption from the gut which is mainly by passive diffusion (Başaran Bacanlı and Başaran 2017).

A systematic review and meta-analysis by Cheng et al. (2017) examined the evidence for tomato and lycopene supplementation. They concluded that interventions using tomato supplement were associated with significant reductions in LDL-cholesterol ($p=0.006$) and improved endothelial function when measured by flow-mediated dilation. In addition, they found giving lycopene supplement reduced systolic blood pressure by 5.66 mmHg ($p=0.002$).

It seems reasonable to suggest, that any antioxidant and anti-inflammatory agent could in theory at least, be protective against multiple sclerosis.

Oat based cereal

In the current study, multiple sclerosis cases ate significantly less oat-based cereal than controls, and in a dose response manner. It is possible that some of these patients have disease associated diarrhoea or constipation. Hinds (1990) conducted a study of 280 multiple sclerosis cases and suggested that about 43% had constipation and 51% had suffered from faecal incontinence at least once in the previous 3 months. He suggested that 68% of the multiple sclerosis cases he studied had some form of bowel dysfunction. Perhaps the patients in our study were reluctant to eat high fibre food in case it affected their bowel function. Eating oats would often be recommended to improve bowel function because of the soluble fibre content. However, there is no evidence to support this. Nor was any literature found to suggest that bowel function in multiple sclerosis cases might be made worse by eating oat-based cereal. Indeed, The US National Multiple Sclerosis Society suggests eating a high fibre diet to help bowel symptoms. In the present study there were no instances when a multiple sclerosis case indicated that they deliberately avoided oat-based cereals.

There were no papers found to link an association between oat-based cereals and multiple sclerosis, but the temporal connection can be made of any food with antioxidant and or anti-inflammatory properties being useful in reducing risk of acquiring multiple sclerosis.

Dried Pulses

Pulses are the dried seeds of legumes such as beans, peas, lentils and chickpeas (Mudryj et al. 2014) and are high in dietary fibre and protein. They are a good source of minerals such as iron, calcium, magnesium, zinc and potassium. They contain vitamin C and folate. A systematic review of 8 controlled feeding trials by Jayalath et al. (2013) concluded that they significantly reduced blood pressure in people with and without hypertension. In this study when compared with control subjects, multiple sclerosis cases reported eating significantly less dried pulses. Some people with multiple sclerosis can have bowel problems. This can take the form of constipation or diarrhoea. It is well known that eating beans for example, can cause flatulence (Messina 2014), and it is possible that multiple sclerosis patients avoid dried pulses for this reason. The long cooking time (Goyal et al. 2018) that pulses need may put some people off using them, but this should be similar for control subjects as well as cases. The preparation of dried pulses usually takes two steps. Soaking for about 24 hours is followed by cooking for between 15 minutes and 2 hours (Margier et al. 2018). It is possible that ill or disabled multiple sclerosis patients cannot be bothered with this process. Canned and jarred pulses would overcome these problems and it would have been interesting to ask an additional question about the reason for people not eating pulses. Pulses are an important component of the Mediterranean diet as an important protein source in place of meat, which is recognised as a healthy dietary option (Willett et al. 1995). However, there is a paucity of studies involving consumption of pulses and patients with multiple sclerosis. Awika et al. (2018) suggest pulses and whole grains have health benefits alone and have a synergistic effect when consumed together. The "foods avoided" question highlighted one case who avoided pulses (without explanation) but this is unlikely to affect the result that cases ate less pulses than control subjects. There is no medical explanation why this occurred.

Beetroot

As well as containing fibre and vitamins, beetroot contains betalain pigments which have been shown to display antioxidant and anti-inflammatory properties (Clifford et al. 2015). Beetroot has been suggested as protective against oxidative stress–related disorders such as cardiovascular disease and neurodegenerative disorders (Vulić et al. 2013). However, betalains have poor bioavailability (Lechner and Stoner 2019) and while betalains have in theory the ability to scavenge free radicals, the bioavailability issue has cast doubt on their usefulness at present although it seems plausible that

beetroot could be neuroprotective against multiple sclerosis. In the age-matched conditional regression analysis, cases reported eating significantly less beetroot compared with controls. As far as is known, there have been no case-control studies in the literature suggesting lower beetroot consumption in multiple sclerosis cases compared with controls.

One serious side effect of eating large amounts of beetroot are oxalate kidney stones in people prone to kidney stones. It is not known if those with multiple sclerosis in this study actively refrained from eating beetroot for this reason, but it seems unlikely. A study by Ganesan et al. (2017) suggests that compared with matched controls, patients with multiple sclerosis were significantly less likely to have calcium oxalate monohydrate stones (39% vs 64%, $P < 0.001$) and more likely to have calcium phosphate stones (42% vs 15%, $P < 0.001$) and struvite stones (8% vs 3%, $P = 0.03$). It might be speculated. multiple sclerosis cases in this study had misread this article or been misinformed by a doctor who had read the article that eating beetroot led to "kidney stones" in multiple sclerosis. However, the answers from the "foods avoided" question gave no indication that multiple sclerosis patients deliberately avoided beetroot.

Refined breakfast cereal

Multiple sclerosis cases reported eating significantly less refined breakfast cereal compared with controls. It would seem counter intuitive that eating less refined breakfast cereal would increase the risk of acquiring multiple sclerosis and the data should not be interpreted in this way.

There is some evidence from the "foods avoided" question that some cases have tried to eat less fried and "unhealthy" food although none indicated that they were avoiding refined breakfast cereal, which is regarded by some as "less healthy" than unrefined breakfast cereal. Refined cereal has been modified by grinding or sifting to removal the bran and germ. This adds to the shelf life but results in losses of many "healthy" components that in some countries it has been deemed necessary to replace certain lost components through mandatory fortification (Mann 2017). It is possible that cases have deliberately reduced the consumption of refined breakfast cereal and switched to the "healthier" whole grain breakfast cereal. However, there are no data from this study to support this suggestion.

Onions

The literature suggests that all onions have antioxidant properties (Borah and Banik 2018) with red onions containing the highest amount of antioxidant compounds, in particular flavonoids. They are also thought to have anti- cancer and anti-inflammatory properties (Roldan et al. 2008). Since multiple sclerosis is an inflammatory disease it is possible to make a temporal connection with any anti-inflammatory compounds that may be protective and that there might be health benefits of consuming onions might be protective against multiple sclerosis When compared with controls, multiple sclerosis patients ate significantly less onions than control subjects, in a dose response manner, although the number of participants who did not eat onions was very small and so statistical calculations should be viewed with caution. One possible explanation is that cases may have been advised against eating onions by a doctor or dietician. However, there are no documented reasons why someone would advise this, and there is no indication in the literature to suggest any interactions between consumption of onions and multiple sclerosis medications.

6.3 Smoking

Smoking is a recognised potential confounder in the study of cardiovascular disease, which may have a similar inflammatory pathogenesis to multiple sclerosis. Smoking fulfilled part of condition 1 of being a confounder in this study (a confounder is defined in Chapter 2 page 26). That is, smoking is shown to be associated with multiple sclerosis (Gross and Lublin 2017). However, it is more difficult to associate smoking with eating a Mediterranean diet. It could be argued that a person who follows an unhealthy lifestyle choice (smoking), is less likely to follow a healthy dietary choice (the Mediterranean diet). To determine if smoking was a confounder in the current study, condition 2 was applied as described in Chapter 2. Adjustments were made statistically to determine if smoking was distributed unequally among the groups being compared. In the study, current smoking incidence was high in both cases and control subjects but was significantly less amongst cases than control subjects. This might give the impression that current smoking might be in some way be protective. For example, the odds ratio was OR 0.32 (0.20, 0.52) p**<0.001** (see Appendix A). This suggests that cases were 68% less likely to be current smokers than were control subjects. There is a plausible reason for this result. On being diagnosed, many multiple sclerosis patients may have given up smoking. This is suggested with the "ever-smoked" (ie current plus former smokers) result, where there was no statistically significant difference in the numbers who had ever

smoked between cases and controls. Unfortunately, there were no data collected in the questionnaire as to when former smokers stopped smoking in relation to their diagnosis of multiple sclerosis. To summarise, current smoking was high amongst both cases and control subjects but was higher amongst control subjects. When "ever-smokers" were compared, there was no statistically significant difference between either group. When the Medscore diet score was adjusted for smoking status or exposure to smoke, there were no differences in the odds ratio. So, for this study smoking was not a confounding factor.

6.4 Demographic characteristics

Summary

The definition of a confounding factor is described and discussed in chapters 1 and 4 and defined in chapter 2 page 26. Although there were several potential confounding factors, the only confounding factor found to be significant in this study was age. Age was associated with both the risk factor of interest and the outcome, and it was distributed unequally between the groups being compared. It was not an intermediary step in the pathway from the exposure of interest to the outcome of interest, so age fulfilled the three conditions of being a confounding factor.

Median age was found to be significantly different between cases and control subjects. Adjustment was therefore made for age in the logistic regression analysis. Obviously, the age-matched conditional regression analysis removed age as a confounding factor.

Condition 1 of the "definition of a confounder" requires the confounding factor to be associated with both the risk factor of interest and the outcome. Skin type and previous diseases do not fulfil this condition. While both variables are possible risk factors of multiple sclerosis, neither can be linked temporally with the Mediterranean diet, so have been removed from the discussion.

Condition 2 requires that the confounding variable is distributed unequally among the groups being compared. Other potential confounders such as sex, educational attainment, were tested as adjustments in the Medscore diet score but were found not to be confounding factors as they had no or little effect on the Medscore diet score.

6.4.1 Sex/Gender

While the variable “sex” fulfilled condition 1 of being a confounder, that is, it was associated with the outcome of interest (the sex ratio worldwide is about 3:1, women to men) and it is plausible that females could eat a different diet to males, it did not fulfil condition 2 in this study. To satisfy this condition, the distribution of the variable would need to be unequal between cases and controls. In this study, that was not the case. Females were about 2/3 of both cases and control subjects. With this in mind, the discussion on sex has been omitted.

6.4.2 Age

The mean age of the multiple sclerosis cases was 46.8 years (SD 13.7) while the mean age of the control subjects was 32.4 years (SD 12.8). The age distribution of the control subjects was not uniform, being skewed towards the younger age range, whereas the ages of cases were normally distributed.

Questionnaires were collected from cases and controls simultaneously by convenience sampling and the mean (then median) age of each group determined afterwards at the analysis stage. With hindsight, more older controls could have been recruited using a more targeted approach if the cases had been recruited first and analysis started. However, the time restrictions and a delay in getting case information for this study prevented this.

The average age at diagnosis of multiple sclerosis in Cyprus is 35 years, according to the principal consultant at the Cyprus Institute of Neurology (Personal communication) but no accurate data exist as there is not a multiple sclerosis registry or universal computerised hospital records system yet in Cyprus.

The age of cases ranged from 19 to 81 years while the range for control subjects was similar, ranging from 18 to 87 years. Since there were over 700 controls, it would have been possible to remove large numbers of young controls from the analysis to bring the median age of controls to nearer 47 years.

However, *a priori*, age was considered a potential confounding factor, and the initial analysis confirmed that this was the case, so it was decided to adjust all the logistic analysis for age statistically.

6.4.3 Socioeconomic status

Socioeconomic status was regarded as a potential confounding factor since it has been suggested as a risk factor for multiple sclerosis in a number of previous studies, although the evidence is conflicting (Leibowitz et al. 1966; Kurtzke and Page 1997; Berg-Hansen and Celius 2015). Low socioeconomic status and poor sanitary conditions have been suggested as being protective against multiple sclerosis but was shown to be statistically significant only when living conditions were well below average (Zilber and Kahana 1996). However, another study found that low socioeconomic status increased the risk for multiple sclerosis in certain ethnic groups such as black and white men and white women (Briggs Green and Weintraub 2015).

Cyprus has the second highest number of people in Europe who have completed tertiary education (The Republic of Cyprus Statistical Service 2017).

In this study, there were no statistical relationships between housing status, marital status, highest qualification obtained and the risk of being a multiple sclerosis case or not. When the Mediterranean diet score data were adjusted for socioeconomic status there were no confounding effects observed.

6.4.4 *Body Mass Index and Body Mass Index categories*

There is strong evidence from observational studies to suggest that obesity is a risk factor for multiple sclerosis (Correale Farez and Gaitán 2017; Alfredsson and Olsson 2018) and meta-analysis supports this (Liu et al. 2016). In this study, which relied on self-reported weight and height, obesity was more common in cases than control subjects (13% compared with 9.5%). As can be seen from the results in chapter 5, cases had a higher mean BMI than control subjects which was statistically significant. However, there was no statistically significant difference between groups when body mass index categories were compared or when obesity data were adjusted for age.

Self-reported weight may be lower than that measured using accurate scales and similarly self-reported height higher than that determined using a measure (Gorber et al. 2007). It is also likely that reporting differed between males and females (Birrell Pearce Francis and Parker 2005). This could make the BMI values calculated for subjects in this study lower than the true value. However, since there is no evidence that self-reporting would be different between cases and control subjects, the comparison is still valid.

It is possible that the effect of the disease process in multiple sclerosis could reduce mobility and hence increase weight gain as the disease advances. It is also possible that

the median age difference between cases and controls could partly explain the mean BMI difference as older people tend to put on weight with age as activity/exercise reduces with age. This is more likely than the minimal effect of slightly slower metabolism observed in multiple sclerosis (Godard 2016).
When the Mediterranean diet score data were adjusted for BMI, there were no confounding effects observed.

6.5 Strengths and limitations of the study

6.5.1 *Strengths*

The study design is considered a strength and followed standard practice. For example, case-control studies are appropriate for rare diseases and multiple sclerosis is a rare disease. To the author's knowledge this is the first known case-control study examining the relationship between adherence of the Mediterranean diet and the risk of acquiring multiple sclerosis in Cyprus, and indeed Europe. Other than one case-control study in Iran, it is only the second known study investigating possible relationships between adherence to the Mediterranean diet and risk of multiple sclerosis in the world.
Using two main methods of data analysis is also considered a strength. The logistic regression analysis with adjustments for potential confounding factors such as age and sex, is a strength. The age-matched analysis was carried out because it avoided a major bias, which would have been a major limitation. When the age-matched analysis was incorporated into the study design, the study became stronger. The analyses using both logistic regression and conditional logistic regression are robust approaches. The study used a validated FFQ based on the Epic-Norfolk FFQ which has been used in many circumstances. The questionnaire was self-administered which avoids interviewer bias. Participants were allowed to answer food questions on a continuum for example a food item might be eaten daily, weekly or monthly. All answers were converted to weekly scores. Another strength is that a local nutritional expert provided information to support the local context of the study. The local expert helped ensure that foods typical of Cyprus were included in the FFQ and that English brands that are not found in Cyprus were identified and excluded. The FFQ was also translated by a professional translator into Greek and another translator translated that Greek version back to English to ensure accuracy of translation. The final FFQ was printed with every question in both English

and Greek. The questionnaire was relatively short and could be completed without any help in about 15 - 20 minutes. Using a single investigator can be time consuming, but it prevents potential errors associated with multiple investigators such as inputting data differently. Another strength is that the study is reproduceable. For example, the FFQ could be used on another Greek speaking island such as Crete (with appropriate ethics approval). Another strength is that there was clear case definition. Cases had a consultant diagnosis using the McDonald criteria revised 2107. Furthermore, the STROBE guidelines for observational studies were followed for this case-control study.

6.5.2 *Limitations*

One potential limitation was control selection. It was not possible to assess to what extent controls were representative of the background population. While every effort was made to collect control data from across the island, it was not possible to ensure this was representitive. However, case and control demographics were not statistically different in the analyses.

The recruitment of cases and control subjects might have suffered from selection bias. Due to the small number of cases on the island, and the way they could be contacted because of the lack of a registry, cases were selected by convenience sampling in hospital clinics. This could be a limitation since some multiple sclerosis cases may be so unwell that they find regular attendance at a clinic more difficult and other multiple sclerosis cases might be in a phase of remission so don't feel they need attending. Control subjects were also selected by convenience sampling because of the timescale of the study as mentioned in the methods chapter. Like other case-control studies, recall bias was a possible limitation. It is possible that cases and controls recalled their diets differently. In multiple sclerosis, cognitive impairment occurs late in the disease so this should not have been a problem in the current study although data on time since diagnosis was not collected. The aim at case selection was to avoid enrolling patients who could not make an informed decision about participation and consent. However, it was not possible to measure cognition, and it is possible that cases were less able to recall dietary details than controls. It is also possible that cases altered their diet upon diagnosis or answered questions differently to the way they would before diagnosis. The low numbers of cases

who answered the questions on “foods avoided” and “foods eaten deliberately because of the disease”, tends to suggest this was not the case.

The use of any score to assess Mediterranean diet adherence as a method of assessing a dietary eating pattern is limited by subjectivity (Sofi 2008) and comparisons with any of the other 48 scores available is difficult. Another weakness is that only a comparison of “Mediterranean diet adherence” or “foods eaten” between these cases and control subjects was possible, since in this study, no measurements of nutrient intake were made. Comparisons to other studies can only be made in general terms. In this context reverse causation is a possible limitation. This when the disease (outcome) increases the likelihood of the exposure. For example, multiple sclerosis patients might avoid a food because of their condition.

The problems of multiple testing also require consideration. When multiple simultaneous tests occur in a study like the current study, each test has the potential to produce a significant result alone. As more tests (in this study 100s) are analysed, it becomes increasingly likely that cases and control subjects will differ due to random sampling error alone. Type 2 errors (or false positives) can occur. Corrections for this potential error were not performed in this study, since the number of hypotheses were small (Streiner and Norman 2011).

6.6 Conclusion and Recommendations

The null hypothesis in this study was: multiple sclerosis cases when compared with control subjects, have neither a higher nor lower Mediterranean diet score. Since cases had a lower Mediterranean diet score and this was statistically significant, it was possible to reject the null hypothesis and accept the alternative hypothesis. The Mediterranean diet is a dietary pattern high in plant-based foods and fruit, while low in dairy and red meat products. There is evidence to suggest the Mediterranean diet is a healthy diet which seems to be protective against cardiovascular disease and colorectal cancer. This current study adds additional evidence that this healthy dietary pattern is lower in multiple sclerosis cases compared with control subjects. This raises the question “Can a diet such as the Mediterranean diet, be a useful intervention for primary prevention of multiple sclerosis?” Future intervention studies might help answer this.

The current study also examined whether food groups within the Mediterranean diet pattern were associated with multiple sclerosis risk. In cardiovascular disease and

colorectal cancer research, there has been a shift away from studying food groups and individual foods, towards diet as a whole. This might be due to a belief that it is the synergistic effect of the components of the diet that are beneficial. This may well be true, but this study found that certain food groups within the Mediterranean diet were consumed less by cases than by control subjects. For example, cases ate less vegetables, ate less legumes, ate less whole grain food and drank less alcohol than control subjects. In addition, cases ate more dairy products than control subjects. Cases also ate more red meat and products than control subjects, but this difference was not statistically significant. Within food groups some individual foods were of interest as cases consumed less grapefruit, tomatoes, oat-based cereal, dried pulses, beetroot, onions and refined breakfast cereal than control subjects. These food groups and individual foods warrant further investigation in the future. However, it is likely that some reverse causation exists. It was not possible to determine where this was occurring, although the discussion of individual foods highlights some potential areas.

The results from this case-control study suggest patients with multiple sclerosis eat a dietary pattern less like the Mediterranean diet and a vegetable-based diet is less likely to be eaten by multiple sclerosis patients than controls. It is recommended that a plant-based diet be considered as a primary prevention measure subject to future intervention studies.

6.6.1 *Suggestions for future research*

Other Mediterranean diet scores

As stated earlier, there are many other Mediterranean diet scores in existence (Zaragoza-Martí et al 2018). It will be possible to use the same data collected from this study and derive other Mediterranean diet scores by only slightly modifying the information already at hand. If similar results were obtained using different Mediterranean diet scores, this would add weight to the view that a Mediterranean type diet is protective against multiple sclerosis. If contradictory results were obtained, then it would cast doubt on this idea.

Large cohort studies

Large cohort studies can add valuable information to the study of a rare disease such as multiple sclerosis. The Oxford Centre for Evidence-based Medicine (2011) suggests that the level of evidence of well-designed large cohort studies is Level II or III depending on the effect. This is more powerful evidence than a case-control study which is considered Level IV. However, following large cohorts of patients forward over long periods of time

can be challenging and expensive. Unfortunately, studies of this type have a long timescale and attrition is often high. The numbers of multiple sclerosis cases needed overall would be very large.

Dietary intervention studies

Randomised intervention clinical trials can provide more powerful evidence (Level II) than cohort studies and can be used to imply causation. Dietary intervention studies can be full studies or proof of concept studies. In the latter, as it possible to design a relatively short study that involves less patient recruitment. A group of perhaps 100 multiple sclerosis cases could be randomly allocated to two groups, one group who would receive a Mediterranean type (plant-based) diet and the second group who would consume a "Western" or control diet. Unfortunately, attrition might be high in such a study, as it is difficult to get people to adhere to a diet for a long period. Yearly MRI results (among other measures) would be needed for comparison to see if there were differences in new lesions and disease progression.

Principal components analysis (PCA)

Principal components analysis (PCA) is a statistical method of converting observations of variables that may be correlated into linearly uncorrelated variables called principal components. For example, with the current data food patterns may be empirically derived. Indeed, this method was considered and started, but abandoned due to time constrains.

6.6.2 *Relevance of the study to health planning in Cyprus.*

It is uncertain the exact cost of the health treatment for a multiple sclerosis patient in Cyprus, but in a UK study, the annual mean cost per patient varied with disease severity from 11,400 GBP for mild cases to 36,500 GBP for severe cases (Kobelt et al. 2017). Cyprus has a population of about 850,000 and the estimated prevalence of the disease is 198 per 100,000 (Charalambidou et al. 2016.) This is far higher than a previous study in 1997 which estimated 44.5 per 100,000 (Dean et al. 1997) and is much higher than the rest of Europe at this latitude. If it were assumed that Cyprus were to have 1600 multiple sclerosis patients needing treatment costing 11,000 Euros per year, this would equate to an annual cost of over 17 million Euros.

The potential for severe disability, the reduction in quality of life and the financial burden to the individual and the state, provides the impetus to find ways of reducing incidence in

both Cyprus and elsewhere. With this in mind, modifying modifiable risk factors such as diet could lead to health benefits for the individual Cypriot and reduce the financial burden to the state. So far, however, the evidence is not clear enough to warrant widescale dietary interventions, so more research is needed with perhaps a registry and high-quality data sources developed.

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The Appendices

Appendix A

This section deals with the results of the unmatched data.

Descriptive results

There were 127 multiple sclerosis cases with a consultant diagnosis (uptake 21.2%) who returned the questionnaire in the time period of the study (August 31st 2017 to August 31st 2018). During the same period 718 control subjects (uptake 60.2%) returned the questionnaire. A summary of the demographic data for the whole population can be seen in Table 1. Data on gender were available for all cases and controls (n=845). The 127 cases were composed of 83 females and 44 males with approximately a 2:1 female to male ratio in the return of questionnaires. The impact of demographic factors as potential confounders is recorded in the section on the Mediterranean diet score.

Table 1 Summary of demographic information collected. Part1

	Controls (n=718) n (%)	Cases (n=127) n (%)	Total	Chi^2 p-value
Sex				
Men	236 (32.9)	44 (34.6)	280 (33.1)	
Women	482 (67.1)	83 (65.3)	565 (66.9)	p= 0.70
Age				
Median Age	27 years (n=686) IQR	46 years (n=123) IQR	29 years (n=809) IQR	p=< **0.001**
Inter quartile range (IQR)	23-38	37-57	24-43	
BMI Categories				
Underweight	57 (8.3)	8 (6.7)	65 (8.1)	
Healthy	418 (61.1)	60 (50.0)	478 (59.5)	
Overweight	143 (20.9)	36 (30.0)	179 (22.3)	p =0.06
Obese	66 (9.7)	16 (13.3)	82 (10.2)	
Total	684	120	804	
Mean BMI	23.61 SD 5.18	24.61 SD 4.43		
Place of birth				
Nicosia	391 (54.6)	50 (50.0)	441(54.0)	
Limassol	111 (15.5)	19 (18.8)	130 (15.9)	
Larnaca	105 (14.7)	13 (12.9	118 (14.4)	
Famagusta	29 (4.1)	5 (5.0)	34 (41.6)	p = 0.85.
Paphos	11 (1.5)	2 (2.0)	13 (15.9)	
Other eg Greece, UK	69 (9.6)	12 (11.9)	81 (9.9)	
Total	716	101	817	
Place of current residency				
Nicosia	486 (68.0)	62 (56.9)	548 (66.5)	
Limassol	107 (15.0)	26 (23.9)	133 (16.1)	
Larnaca	100 (14.0)	16 (14.7)	116 (14.1)	p = 0.11
Famagusta	11 (1.5)	3 (2.8)	14 (17.0)	
Paphos	6 (0.8)	2 (1.8)	8 (0.1)	
Other eg working abroad	5 (0.7)	0 (0.0)	5 (0.1)	
Total	715	109	824	
Ethnicity				
Greek Cypriot Yes				
No	694 (97.7)	117 (96.7)	811 (97.6)	p = 0.49
Total				
Maternal ethnicity	16 (2.3)	4 (3.3)	20 (2.4)	
Greek Cypriot Yes				
No	710	121	831	
Total				
Paternal ethnicity				p = 0.81
Greek Cypriot Yes	660 (93.2)	113 (92.6)	773 (93.1)	
No				

Total	48 (6.8) 708 674 (95.3) 33 (4.7) 707	9 (7.4) 122 114 (94.2) 7 (5.8) 121	57 (6.9) 830 788 (95.2) 40 (4.8) 828	p = 0.60
Housing status				
Owned	303 (43.3)	86 (71.1)	389 (47.4)	
Rented	328 (46.9)	25 (20.7)	353 (43.0)	p=0.98
Other	68 (9.7)	10 (8.3)	78 (9.5)	
Total	699	121	820	
Marital status				
Cohabiting	58 (8.2)	4 (3.2)	62 (7.5)	
Divorced	23 (3.3)	8 (6.5)	31 (3.7)	
Married	232 (32.9)	83 (66.9)	315 (38.0)	p=0.98
Single	384 (54.5)	23 (18.6)	407 (49.1)	
Widowed	8 (1.1)	6 (4.8)	14 (1.7)	
Total	705	124	829	
Highest qualification				
No qualification				
Apolyterium/ GCSE	68 (10.7)	11 (9.9)	79 (10.6)	p=0.92
BA/BSC	263 (41.2)	47 (42.3)	310 (41.4)	
Masters/ PHD	165 (25.9)	31 (27.9)	196 (26.2)	
Total	142 (22.3)	22 (19.8)	164 (21.9)	
	638	111	749	

In the logistic regression analysis data, from 127 cases and 718 controls were used. There were no cases or controls with every single question answered in full. In the age-matched analysis, 119 cases had provided age, and these were matched with 119 controls as described in Chapter 4.

Details of how missing data were treated appear in the methods section in Chapter 4 and the discussion of the impact on the study of missing data is dealt with in the discussion section in Chapter 6.

All missing data in the demographic factor section of the questionnaire were left blank when uploaded to the statistical software. As an example of missing data and available data, the logistic regression analysis of % available data in the demographics section is shown in table 2

Table 2 Summary of demographic information (% data available) used in logistic regression analysis

	Controls n (% data available) (n=718)	Cases n ((% data available) (n=127)
Sex Men Women	100% 236 (100) 482 (100)	100%
Age	686 (95.5)	123 (96.9)
BMI Categories Total	684 (95.3)	120 (94.5)
Place of birth Total	716 (99.7)	101 (79.5)
Place of current residency Total	715 (99.6)	109 (85.3)
Ethnicity Total **Maternal ethnicity** Total **Paternal ethnicity** Total	710 (98.9) 708 (98.6) 707 (98.5)	121 (95.3) 122 (96.1) 121 (95.3)
Housing status Total	631 (87.9)	111 (87.4)
Marital status Total	705 (98.2)	124 (97.6)
Highest qualification Total	638 (88.9)	111 (87.4)

There was no significant difference between the missing data of cases and controls, except for place of birth, which didn't impact on the Mediterranean diet results.

Age was recorded for 809/845 people (95.74%), with similar percentage of cases and controls providing this information. Age ranged between 18 and 87 years old for controls and 19 and 81 for cases. The mean age of the cohort was 34.7 years (SD 14) and the median age 29 years (IQR 24-43). The age of the controls was not normally distributed, being skewed towards the younger ages, so the median was used.

The whole cohort median age was 29 years, but the median age of cases was 46 years and of controls 27 years. The results showed that age was significantly different between cases and controls ($p<0.001$).

Of the four cases who were included as "Greek Cypriots" but declared themselves as "other", two were born outside Cyprus but gave both parents as "Greek Cypriots". The other two both gave both parents as "other" but neither filled in their place of birth. The sixteen controls who were included as "Greek Cypriots" but declared themselves as "other", gave both parents as "other" or left the answer blank. They stated they were born outside Cyprus or left the answer blank.

All other categories in the demographic data showed no statistically significant association between cases and controls.

Analysis of the demographic information showed that only age was statistically different between cases and controls in the logistic regression analysis. The effect of demographic data results are shown as potential confounders in the Mediterranean diet score analysis later.

The Mediterranean diet

Descriptive table and summaries of a Mediterranean diet score adapted from the MedDietScore

A Mediterranean diet score (Medscore) derived from the MedDietScore (Panagiotakos et al, 2007) scoring system was used to assess adherence to the Mediterranean diet as described in the methodology chapter. Each of 11 food categories was given a score. The maximum total Mediterranean diet score was the sum of the 11 components (10x5) +3(olive oil) = 53. The minimum and maximum scores for the cohort were 12 and 50, respectively. Cases had a slightly lower mean score of 33.3 (SD 5.24) and ranged from 17 to 46 while the mean score for the control group was 33.9 (SD 5.74) and ranged from 12 to 50. There was no statistical significance between the mean Medscore of cases and controls.

Defining "dose response"

After assessing if cases and controls were exposed to a variable in a binary manner, for example ate a food or not, a dose response association was calculated. With a "dose response" association, for every step increase in the exposure of interest, there is an accompanied stepwise increase or reduction in the likelihood of the outcome (for example having multiple sclerosis).

The mean medscores of the 11 food categories were calculated for the whole cohort and separately for the age-matched cohort. These appear in Table 3

Table 3 Mean medscores of the 11 food categories in the analysis using 718 controls and 127 cases

	Controls (n=718) Mean (SD)	Cases (n=127) Mean (SD)
Alcohol	3.49 (2.28)	2.44 (2.51)
Red meat and products	2.36 (1.77)	2.85 (1.49)
Poultry	4.45 (1.28)	4.83 (0 .71)
Fish	2.38 (1.41)	2.58 (1.27)
Non-Refined cereals	2.18 (1.21)	1.97 (1.02)
Potatoes	3.38 (1.39)	2.99 (1.34)
Dairy products	3.62 (1.57)	4.12 (1.31)
Fruit	4.09 (1.20)	4.27 (1.19)
Vegetables	2.99 (1.34)	2.77 (1.27)
Legumes	2.94 (1.23)	2.61 (1.07)
Olive oil	2.10 (1.38)	1.89 (1.46)

Table 4 The Total Medscore in relation to multiple sclerosis. Unadjusted, and with adjustments using Logistic regression analysis

	Total Med Diet Score OR [95% CI] p
Dose response unadjusted	0.98 (0.95, 1.01) 0.26
Dose response Adjusted for age	0.96 (0.93, 1.00) **0.04**
Dose response Adjusted for sex	0.98 (0.95, 1.01) 0.25
Dose response Adjusted for age and sex	0.96 (0.93, 1.00) **0.04**

Statistically significant results were obtained. For each unit of the Total Medscore in the logistic regression analysis adjusted for age, there was a 4% reduction in the likelihood of the outcome (multiple sclerosis).

Results of demographic information as potential confounders are shown in table 5 below.

Table 5 Logistic regression analysis Dose response of the total Medscore with adjustments of potential demographic confounding factors

	Total Med Score OR [95% CI] p
Unadjusted	0.98 (0.95, 1.01) 0.26
Adjusted for age	0.96 (0.93, 1.00) **0.04**
Adjusted for sex	0.98 (0.95, 1.01) 0.25
Adjusted for age and sex	0.96 (0.93, 1.00) **0.04**
Adjusted for age and Body mass scale	0.96 (0.92, 1.00) 0.06
Adjusted for age and Ethnicity	0.96 (0.93, 1.00) 0.06
Adjusted for age and mother's ethnicity	0.96 (0.92, 1.00) **0.04**
Adjusted for age and father's ethnicity	0.96 (0.92, 1.00) **0.04**
Adjusted for age and place of birth	0.97 (0.93, 1.01) 0.10
Adjusted for age and current residency	0.96 (0.92, 1.00) **0.04**
Adjusted for age and level of education	0.96 (0.93, 1.00) 0.07
Adjusted for age and highest qualification	0.96 (0.92, 1.00) **0.05**
Adjusted for age and marital status	0.96 (0.92, 1.00) **0.02**
Adjusted for age and housing status	0.96 (0.92, 1.00) **0.03**

The results of alcohol consumption as potential confounders are shown in the Table 6 below.

Table 6 Logistic regression analysis of the dose response of the total Medscore with adjustments for alcoholic drink consumption as potential confounding factors

	Total Med Score OR [95% CI] p
Unadjusted	0.98 (0.95, 1.01) 0.26
Adjusted for age	0.96 (0.93, 1.00) **0.04**
Adjusted for sex	0.98 (0.95, 1.01) 0.25

Adjusted for age and sex	0.96 (0.93, 1.00) **0.04**
Adjusted for age and beer, cider per week	0.99 (0.95, 1.03) 0.49
Adjusted for age and units of spirits per week	0.97 (0.92, 1.00) 0.07
Adjusted for age and fortified wines per week	0.96 (0.93, 1.00) **0.05**
Adjusted for age and wine per week	0.96 (0.92, 1.00) **0.03**
Adjusted for age and Red wine per week	0.96 (0.92, 1.00) 0.07
Adjusted for age and White wine per week	0.96 (0.92, 1.00) 0.10
Adjusted for age and Rose wine per week	0.97 (0.93, 1.00) 0.07

The Medscore does not change by more than 10% when adjusted for alcohol, so the consumption of alcoholic drinks were not confounding factors.
Food group Medscores were analysed and are shown in Table 7.

Table 7 Dose response of The Mediterranean Diet Scores of food groups

Total Med Score				
	Dose response unadjusted OR [95% CI] P	**Dose response adjusted for age OR [95% CI] P**	**Dose response adjusted for sex OR [95% CI] P**	**Dose response adjusted for age and sex OR [95% CI] P**
Vegetables	0.89 (0.77, 1.02) 0.10	0.83 (0.70, 0.98) **0.03**	0.89 (0.77, 1.02) 0.09	0.83 (0.70, 0.98) **0.03**
Dairy Products	1.28 (1.11, 1.49) **.001**	1.24 (1.05, 1.46) **0.01**	1.28 (1.10, 1.49) **.001**	1.24 (1.05, 1.47) **0.01**
Alcohol	0.84 (0.78, 0.90) **<0.001**	0.87 (0.80, 0.95) **0.001**	0.84 (0.77, 0.90) **<0.001**	0.87 (0.80, 0.94) <**0.001**
Legumes	0.78 (0.66, 0.93) **0.004**	0.78 (0.64, 0.95) **0.01**	0.78 (0.66, 0.93) **0.004**	0.78 (0.64, 0.95) **0.01**
Non-refined cereals	0.85 (0.72, 1.01) 0.07	0.81 (0.66, 0.98) **0.03**	0.85 (0.72, 1.01) 0.07	0.81 (0.67, 0.98) **0.03**
Olive oil	0.90 (0.79, 1.03) 0.13	0.92 (0.80, 1.07) 0.27	0.90 (0.79, 1.03) 0.13	0.92 (0.80, 1.07) 0.27
Red meat and products	1.18 (1.06, 1.33) **0.004**	1.04 (0.91, 1.18) 0.60	1.19 (1.06, 1.33) **0.003**	1.03 (0.91, 1.18) 0.62
Poultry	1.49 (1.15, 1.94) **0.003**	1.30 (0.98, 1.71) 0.07	1.49 (1.15, 1.94) **0.003**	1.30 (0.98, 1.71) 0.07
Fish	1.11 (0.97, 1.27) 0.13	1.13 (0.97, 1.30) 0.12	1.11 (0.97, 1.27) 0.14	1.13 (0.97, 1.31) 0.11
Potatoes	0.81 (0.71, 0.94) **0.004**	0.86 (0.74, 1.01) 0.07	0.81 (0.71, 0.93) **0.004**	0.86 (0.74, 1.01) 0.07

Fruit	1.14 (0.96, 1.35) 0.13	0.94 (0.78, 1.13) 0.49	1.14 (0.96, 1.35) 0.14	0.94 (0.78, 1.13) 0.50

In the logistic regression analysis adjusted for age, the Medscore scores for consumption of vegetables, alcohol, legumes and potato were significantly negatively associated with being a multiple sclerosis case. This association was found to follow a dose response for vegetables, alcohol and legumes. The Medscore for non-refined cereals when adjusted for age was significantly negatively associated with being a multiple sclerosis case and followed a dose response. Medscores for red meats and meat products as well as poultry meat were significantly positively associated with being a multiple sclerosis case but did not follow a dose response. In contrast, the Medscore for dairy products was significantly positively associated with being a multiple sclerosis case and this score followed a dose response when adjusted for sex.
In summary, a statistically significant association existed between certain food groups that contributed to the Mediterranean diet score.
The individual foods were examined in a binary association (ate this food or not) using logistic regression unadjusted and for a dose response for the "dose response," frequency of consumption was used.

Table 8 Summary of individual foods with a statistically significant dose response and the binary logistic regression results unadjusted, adjusted for age adjusted for sex and adjusted for age and sex

Food	Binary logistic regression Unadjusted OR [95% CI] P	Binary logistic regression Adjusted for age OR [95% CI] P	Binary logistic regression Adjusted for sex OR [95% CI] P	Binary logistic regression Adjusted for age and sex OR [95% CI] P	Dose response Adjusted for age and sex OR [95% C.I.] P
Grape Fruit	0.35 (0.18, 0.65) **0.04**	0.46 (1.05, 1.08) **0.04**	0.35 (0.18, 0.65) **0.001**	0.46 (0.22, 0.96) **0.04**	0.87 (0.77, 1.00) **0.05**
Tomatoes	0.41 (0.19, 0.92) **0.03**	0.29 (0.11, 0.73) **0.008**	0.41 (0.19, 0.92) **0.03**	0.29 (0.12, 0.73) **0.01**	0.96 (0.92, 1.00) **0.05**
Onions	0.43 (0.18, 0.99) **0.05**	0.32 (0.12, 0.86) **0.02**	0.43 (0.18, 0.99) **0.05**	0.32 (0.12, 0.86) **0.02**	0.90 (0.82, 0.98) **0.02**
Refined breakfast cereal	0.19 (0.11, 0.34**) < 0.001**	0.31 (0.16, 0.61) **0.001**	0.19 (0.11, 0.34**) < 0.001**	0.31 (0.16, 0.61) **0.001**	0.90 (0.82, 0.99) **0.04**
Dried Pulses	0.23 (0.13, 0.41) **<0.001**	0.36 (0.18, 0.70) **0.003**	0.23 (0.13, 0.41) **< 0.001**	0.36 (0.18, 0.70) **0.003**	0.84 (0.72, 0.99) **0.04**
Beetroot	0.37 (0.20, 0.68) **0.002**	0.44 (0.21, 0.92) **0.03**	0.36 (0.19, 0.68) **0.001**	0.97 (0.62, 1.51) 0.89	0.75 (0.59, 0.95) **0.02**

Grapefruit, tomatoes, onions, refined breakfast cereal, dried pulses and beetroot consumption were significantly negatively associated with being a multiple sclerosis case. This association was found to follow a dose response. That is, a linear inverse association existed between the frequency of consumption of these foods and the presence of multiple sclerosis. Whole meal pasta and tofu consumption were significantly negatively associated with being a multiple sclerosis case but did not follow a dose response. Consumption of some individual foods such as fish and meats were significantly positively associated with multiple sclerosis. However, there were no dose response associations with any of these positively associated foods.

Analysis of smoking data

Current smoking was high among cases (30/127) 23.6% and controls (352/718) 49.0% but cases were less likely to be current smokers compared with controls and this was statistically significant in all analyses.

Exposure to smoke was high among cases and controls. There were 51 of 127 multiple sclerosis cases who were regularly exposed to smoke (40.2%), while 54.6% (392 of 718) controls were regularly exposed to smoke. These results included those exposed to smoke from people smoking in the home and at the workplace (as happens despite the smoking law prohibiting this) and social interaction with people who were smoking for example at outdoor cafes and bars. There was a significant association using Pearson Chi^2 test = 9.02 p = 0.003 but there was no significant difference between cases and controls being regularly exposed to smoke with logistic regression analysis adjusted for age and sex, OR 0.85 (0.56, 1.30) [95% CI] p=0.46.

Analysis of alcohol consumption data

The amount of alcohol consumed per week was documented as units per week and is presented in table 9 below.

Table 9 Type of alcohol consumed per week. Chi square test, and Binary logistic regression (drinking or not) analysis

	Pearson's Chi2 test	Binary Unadjusted for age and sex OR [95% CI] P	Binary Adjusted for age OR [95%CI] P	Binary Adjusted for sex OR [95% CI] P	Binary Adjusted for age and sex OR [95% CI] P

Units spirits/week	16.95 0.46	0.47 (0.29, 0.74) **0.001**	0.63 (0.38, 1.04) 0.07	0.46 (0.29, 0.74) **0.001**	0.46 (0.30, 0.72) **0.001**
Fortified wines/week	4.16 0.39	0.17 (0.02, 1.22) 0.08	0.12 (0.02, 0.90) **0.04**	0.17 (0.02, 1.21) 0.08	0.12 (0.02, 0.89) **0.04**
Beer /cider/week	34.32 **0.01**	0.29 (0.18, 0.45**)** **< 0.001**	0.27 (0.16, 0.44) < **0.001**	0.28 (0.18, 0.45) < **0.001**	0.26 (0.16, 0.43) <**0.001**
White Wine	7.53 0.82	0.51 (0.28, 0.91) **0.02**	0.49 (0.26, 0.90) **0.02**	0.51 (0.23, 0.92)**0.02**	0.48 (0.26, 0.90) **0.02**
Rose Wine	13.24 0.21	0.26 (0.12, 0.58) **0.001**	0.34, (0.14, 0.80) **0.01**	0.27 (0.12, 0.58) **0.001**	0.33 (0.14, 0.79) **0.01**
Red Wine	13.80 0.39	0.87 (0.56, 1.34) 0.52	0.76 (0.47,1.22) 0.26	0.86 (0.56, 1.33) 0.49	0.76 (0.47, 1.23) 0.26
Wine/week	5.91 0.97	0.84 (0.57, 1.25) 0.38	0.94 (0.61, 1.45) 0.77	0.84 (0.57, 1.26) 0.40	0.93 (0.60, 1.44) 0.74

In the binary logistic regression analysis adjusted for age and sex, there was a statistically significant association between cases and units of spirits/week, fortified wines/week, beer /cider/week, white wine and rose wine consumed per week and cases of multiple sclerosis. However, there was no statistically significant association with red wine or wine per week and cases of multiple sclerosis.
Cases were around 88% less likely to drink fortified wines and 73% less likely to drink beer/cider. Cases were around 51% less likely to drink white wine and around 67% less likely to drink rose wine (adjusted for age and sex). The numbers of alcohol drinkers were small in both cases and controls.

The results of each alcoholic drink as a potential confounding factor have been described in relation the Medscore. None of the alcohol drinks were found to be potential confounding factors.

Missing Data

Details of how missing data were treated are described in the methods section in Chapter 4 and the discussion of the impact on the study of missing data is dealt with in the discussion section in Chapter 6. In the logistic regression analysis data from 127 cases and 718 controls were

used. There were no cases or controls with every single question answered in full, so decisions were made on how to deal with the missing data.

Cases vs controls

The difference in missing data in cases and controls varied depending on the question. As an example, when asked about foods which were avoided, 359/718 (50%) controls answered compared to 41/127 (32.3%) cases, so for cases there were approximately 18% more data missing for this question. As an example of low response rate, when asked about the name of the salt substitute used, 73/845 (8.6%) of controls answered while 9/127 (7.1%) of cases did. Again, cases had slightly more data missing but the results were closer in this instance.

It was beyond the scope of this thesis to analyse every question in the questionnaire on the basis of available or missing data. However, some general remarks can be made using the demographics section as an example. Available data for some questions were generally high in both cases and controls. For example, gender was known for 100% of cases and controls. Age was known for 95.5% of controls and 96.9% of cases, marital status was answered for 98.2% of controls and 97.6% of cases.

Smoking questions

All of the participants responded to the question if they currently smoke or not or ever smoked or not or were regularly exposed to smoking or not. However, when a respondent said they currently did not smoke and had never smoked, it can be assumed they did not smoke manufactured cigarettes, rolled cigarettes and any type of cigars. Some of these respondents had left the section on ways of smoking blank. It has been assumed that these responses were null rather than blank. All other blanks were recognised and coded as blank responses. As a result, the smoking data are complete in some areas and incomplete in others. When quantities were required, or free text spaces for answers were available, many more blanks appeared. When the answer was yes or no, respondents were more likely to provide an answer. Some questions had considerable amounts of missing data. Where no assumptions could be made, gaps were entered as blanks. For example, “filters used”. About 1/3 of these data were missing.

Alcohol

When asked how much alcohol they consumed per week, 199 respondents reported zero and 2 left the question blank. For all other questions on wine, beer or spirit consumption these 199 people were assumed to drink zero units of all types of alcohol per week.

A Mediterranean diet score and individual foods

A Mediterranean diet score was calculated for all participants. Missing data on individual foods was generally low so it was still possible to calculate a value for each of the categories in the Medscore system.

However, when asked about foods which were avoided, only 400/845 answered (47.3%), leaving 52.7% data missing.

When asked about the amount of food eaten, some questions also had a very poor response. For example, when asked about Souvla eaten, only 50/845 answered (5.9%) so there were 94.1% data missing. Calculations on the amount of Souvla eaten were therefore not attempted.

Another example of a very poor response was the amount of grilled meat eaten. This was answered by 53/845 (6.3%) so there was 93.7% data missing and again no attempt was made to make calculation from these data.

Free text questions

Free text questions generally had poor responses with much missing data. For example, when asked about salt substitute used only 82/845 (9.7%) answered and when asked about herbal remedies taken 136/845 (16.1%) answered.

7.2 Hypothesis testing

The *Null hypothesis tested* was that there is no difference in Mediterranean diet pattern score, or intake of specific food groups, or foods, between multiple sclerosis cases and control subjects.

The results presented in this chapter indicate that the *Null hypothesis* can be rejected, and the alternative hypothesis accepted. That is, there are differences in the Mediterranean diet pattern score, or intake of specific food groups, or foods, between multiple sclerosis cases and control subjects.

Appendix B

Table 1 showing the association of individual foods and the likelihood of being consumed or not by cases, unadjusted. Determined by binary logistic regression. Dose response unadjusted

Food	Binary (ate this fruit or not) OR [95% C I] unadjusted p	Dose response OR [95% C I] unadjusted… p
Fruit		
Apples	0.37 (0.14,1.00) **0.05**	1.00 (0.97, 1.04) 0.89
Avocado	0.30 (0.16, 0.56) **<0.001**	0.97 (0.89, 1.03) 0.25
Bananas	0.52 (0.22, 1.24) 0.14	1.02 (0.99, 1.05) 0.26
Grapefruit	0.35 (0.18, 0.65) **0.001**	0.88 (0.78, 1.00) **0.04**
Grapes	0.32 (0.15, 0.68) **0.003**	0.97 (0.91, 1.02) 0.22
Marrow	0.55 (0.22, 1.40) 0.29	1.01 (0.97, 1.05) 0.80
Melon	0.46 (0.22, 0.94) **0.03**	1.03 (0.95, 1.04) 0.72
Oranges	0.49 (0.22, 1.13) **0.09**	1.00 (1.00, 1.05) **0.50**
Peaches	0.35 (0.17, 0.71) **0.04**	0.99 (0.95, 1.03) 0.68
Pears	0.30 (0.14, 0.67) **0.003**	0.97 (0.93, 1.02) 0.20
Peas	0.54 (0.24, 1.22) 0.14	0.84 (0.71, 0.99) **0.04**
Strawberries	0.40 (0.19, 0.82) **0.01**	1.01 (0.96, 1.03) 0.88
Sweet peppers	0.28 (0.14, 0.57) **<0.001**	0.90 (0.81, 0.99) **0.03**
Tomatoes	0.41 (0.19, 0.92) **0.03**	0.90 (0.96, 1.02) 0.43
Dried fruit	0.34 (0.17, 0.68) **0.02**	1.00 (0.96, 1.03) 0.81
Tinned fruit	0.27 (0.15, 0.46) **<0.001**	0.82 (0.66, 1.01) 0.07
Fish		
Fried fish in batter	3.53(2.12, 5.88) **<0.001**	0.96 (0.86, 1.06) 0.41
Fish fingers	2.99 (1.87, 4.78) **<0.001**	0.91 (0.78, 1.07) 0.25
White fish	2.62 (1.55, 4.43) **<0.001**	0.97 (0.89, 1.06) 0.48
Oily fish	3.50 (1.89, 6.48) **<0.001**	0.98 (0.91, 1.06) 0.68
Shell fish	2.94 (1.77, 4.91) **<0.001**	0.98 (0.91, 1.04) 0.47

Fish roe	3.53 (2.21, 5.64) **<0.001**	1.00 (0.95, 1.06) 0.91
Vegetables		
Beetroot	0.37 (0.20, 0.68) **0.002**	0.81 (0.67, 0.97) **0.02**
Broccoli	0.30 (0.15, 0.63) **0.001**	0.92 (0.84, 1.01) 0.95
Brussels sprouts	0.27 (0.15, 0.49) **<0.001**	0.82 (0.69, 0.99) **0.04**
Cabbage	0.21 (0.11, 0.41) **<0.001**	0.93 (0.87, 1.01) 0.07
Capers	0.42 (0.22, 0.78) **0.01**	0.92 (0.84, 1.02) 0.12
Carrots	0.40 (0.15, 1.06) 0.07	0.98 (0.94, 1.02) 0.32
Cauliflower	0.40 (0.19, 0.82) **0.01**	0.93 (0.84, 1.03) 0.17
Charcoal grilled veg	1.06 (0.13, 8.89) 0.06	0.74 (0.56, 0.98) **0.04**
Leeks	0.28 (0.15, 0.52) **<0.001**	0.90 (0.84, 1.11) 0.27
Onions	0.43 (0.18, 0.99) **0.05**	0.95 (0.90, 1.00) 0.10
Green salad	0.55 (0.22, 1.40) 0.21	0.98 (0.95, 1.02) 0.36
Parsnips	0.26 (0.14, 0.48) **<0.001**	0.84 (0.65, 1.08) 0.17
Spinach	0.34 (0.15, 0.77) **0.01**	0.89 (0.78, 1.01) 0.67
Sweetcorn	0.21 (0.11, 0.43) **<0.001**	0.87 (0.77, 0.98) **0.02**
Watercress	0.29 (0.17, 0.48) **<0.001**	0.69 (0.47, 1.00) **0.05**
Dairy products		
Single cream	0.44 (0.22, 0.85) **0.02**	0.80 (0.61, 1.04) 0.10
Clotted cream	0.35 (0.20, 0.63) **<0.001**	0.76 (0.54, 1.07) 0.12
Low fat yoghurt	0.48 (0.23, 1.00) **0.05**	0.92 (0.84, 1.00) 0.06
Full fat yoghurt	0.48 (0.23, 1.00) **0.05**	0.92 (0.82, 1.03) 0.15
Desserts	0.32 (0.17, 0.60) **<0.001**	0.77 (0.62, 0.96) **0.02**
Cheese	1.06 (0.24, 4.80) 0.94	0.95 (0 .90 1.01) 0.13
Eggs	0.45 (0.16, 1.29) 0.14	0.85 (0.76, 0.95) **0.01**
Quiche	0.56 (0.34, 0.90) **0.02**	0.79 (0.61, 1.04) 0.09
Salad cream	0.27 (0.15, 0.51) **<0.001**	0.93 (0.85, 1.02) 0.13
French dressing	0.28 (0.16, 0.49) **<0.001**	0.97 (0.90, 1.04) 0.36
Butter	0.23 (0.11, 0.50) **<0.001**	0.98 (0.93, 1.04) 0.45
Margarine	0.28 (0.14, 0.56) **<0.001**	1.01 (0.95, 1.06) 0.85

Low fat spread	0.33 (0.17, 0.64) **0.001**	0.98 (0.92, 1.04) 0.47
Very low fat spread	0.31 (0.17, 0.58) **<0.001**	0.94 (0.86, 1.03) 0.16
Red Meat		
Beef	1.80 (0.98, 3.29) 0.06	0.92 (0.83, 1.01) 0.09
Beef burgers	2.15 (1.26, 3.68) **0.01**	0.95 (0.89, 1.03) 0.20
Bacon	2.84 (1.72, 4.69) **<0.001**	0.93 (0.81, 1.05) 0.24
Pork	1.66 (0.84, 3.28) 0.15	0.94 (0.87, 1.02) 0.15
Lamb	3.08 (1.83, 5.19) **<0.001**	0.85 (0.72, 1.00) **0.05**
Ham	2.38 (1.43, 3.98) **0.001**	0.89 (0.80, 1.00) **0.05**
Corned beef	4.25 (2.65, 6.84) **<0.001**	0.77 (0.59, 1.01) **0.05**
Sausages	4.49 (2.60, 7.74) **<0.001**	0.98 (0.89, 1.07) 0.64
Souvlaki	10.45 (1.43, 76.22) **0.02**	0.69 (0.53, 0.89) **0.004**
Souvla	1.96 (0.25, 15.32) 0.52	0.39 (0.21, 0.72) **0.002**
Charcoal grilled meat	1.06 (0.13, 8.89) 0.96	0.66 (0.49, 0.90) **0.01**
Grilled roasted meat	1.48 (0.62, 3.52) 0.38	0.83 (0.74, 0.93) **0.002**
Other foods		
Beansprouts	0.24 (0.13, 0.44) **<0.001**	0.92 (0.82, 1.04) 0.18
Coleslaw	0.29 (0.16, 0.55) **<0.001**	0.97 (0.91, 1.04) 0.36
Garlic	0.29 (0.15, 0.57**)** **<0.001**	0.92 (0.84, 1.01) 0.09
Dried pulses	0.23 (0.13, 0.41) **<0.001**	0.83 (0.70, 0.98) **0.03**
Baked beans	0.47 (0.22, 1.04) 0.06	0.93 (0.83, 1.04) **0.19**
Mushrooms	0.38 (0.16, 0.90) **0.03**	0.85 (0.75, 0.96) **0.01**
Veg. derived Protein		
Tofu	0.29 (0.17, 0.50) **<0.001**	0.26 (0.09, 0.82) 0.02
White Meat		
Chicken	2.28 (0.97, 5.37) 0.06	0.95 (0.90, 1.00) 0.06
Cereal		
White bread	0.43 (0.18, 0.99) **0.05**	0.99 (0.95, 1.02) 0.46

Whole meal Bread	0.32 (0.17, 0.68) 0.002	0.99 (0.96, 1.02) 0.55
Cream Crackers	0.35 (0.19, 0.63) <0.001	0.99 (0.93, 1.05) 0.74
Crispbread	0.44 (0.22, 0.91) **0.03**	1.00 (0.96, 1.05) 0.99
Oat based cereal	0.32 (0.17, 0.61) **<0.001**	0.93 (0.87, 1.00) **0.04**
Refined breakfast cereal	0.19 (0.10, 0.34) **<0.001**	0.85 (0.76, 0.94) **0.002**
Carbohydrates		
Potatoes	0.37 (0.15, 0.92) **0.03**	0.93 (0.85, 1.02) 0.14
Chips	0.49 (0.23, 1.08) **0.08**	0.93 (0.84, 1.03) 0.17
Roast potatoes	0.79 (0.26, 2.38) 0.68	0.87 (0.75, 1.02) 0.08
Potato salad	0.49 (0.23, 1.08) 0.08	0.77 (0.60, 0.99) **0.04**
White rice	0.94 (0.27, 3.28) 0.93	0.87 (0.77, 0.99) **0.03**
Brown rice	0.36 (0.20, 0.63) **<0.001**	0.79 (0.64, 0.98) **0.03**
White green pasta	0.30 (0.14, 0.65) **0.002**	0.86 (0.75, 0.98) **0.03**
Whole meal pasta	0.39 (0.21, 0.74) **0.004**	0.74 (0.59, 0.91) **0.01**
Lasagne	0.32 (0.16, 0.62) **0.001**	0.73 (0.53, 0.99) **0.04**
Pizza	0.38 (0.16, 0.90) **0.03**	0.69 (0.51, 0.94) **0.02**

Table 2 The association of individual foods and the likelihood of being consumed or not by cases, adjusted for age. Determined by binary logistic regression. Dose response adjusted for age.

Food	Binary (ate this fruit or not) adjusted for age OR [95% C I] p	Dose response adjusted for ageOR [95% C I] p
Fruit		
Apples	0.21 (0.07, 0.66) **0.01**	0.99 (0.96, 1.03) 0.68
Avocado	0.37 (0.18, 0.73) **0.01**	0.97 (0.90, 1.06) 0.51
Bananas	0.53 (0.19, 1.52) 0.24	1.03 (1.00, 1.06) 0.09
Grapefruit	0.46 (0.22, 0.96) **0.04**	0.87 (0.77, 1.00) **0.05**
Grapes	0.35 (0.14, 0.85) **0.02**	0.96 (0.9, 1.02) 0.14
Marrow	0.32 (0.10, 0.98) **0.05**	1.00 (0.95, 1.04) 0.82
Melon	0.41 (0.18, 0.97) **0.04**	1.00 (0.95, 1.04) 0.82
Oranges	0.34 (0.14, 0.86) **0.02**	1.01 (0.97, 1.04) 0.78

Peaches	0.35 (0.15, 0.82) **0.02**	0.99 (0.95, 1.04) 0.77
Pears	0.24 (0.09, 0.63) **0.004**	0.97 (0.91, 1.02) 0.22
Peas	0.45 (0.17, 1.21) 0.12	0.92 (0.79, 1.06) 0.24
Strawberries	0.33 (0.14, 0.76) **0.01**	1.01 (0.97, 1.05) 0.72
Sweet peppers	0.28 (0.12, 0.66) **0.004**	0.90 (0.82, 1.00) 0.06
Tomatoes	0.29 (0.11, 0.73) **0.01**	0.96 (0.92, 1.00) **0.05**
Dried fruit	0.47 (0.21, 1.09) 0.08	1.00 (0.95, 1.04) 0.75
Tinned fruit	0.42 (0.22, 0.79) **0.01**	0.85 (0.68, 1.06) 0.14
Fish		
Fried fish in batter	5.31 (2.98, 9.46) **<0.001**	1.01 (0.93, 1.10) 0.78
Fish fingers	3.95 (2.34, 6.67) **<0.001**	0.99 (0.89, 1.10) 0.84
White fish	2.93 (1.65, 5.21) **<0.001**	1.02 (0.94, 1.10) 0.67
Oily fish	3.76 (1.94, 7.28) **<0.001**	1.01 (0.94, 1.09) 0.75
Shell fish	3.95 (2.23, 6.99) **<0.001**	1.01 (0.95, 1.07) 0.75
Fish roe	4.29 (2.56, 7.19) **<0.001**	1.03 (0.98, 1.09) 0.25
Vegetables		
Beetroot	0.44 (0.21, 0.92) **0.03**	0.75 (0.59, 0.95) **0.02**
Broccoli	0.42 (0.18, 0.99) **0.05**	0.97 (0.90, 1.04) 0.39
Brussels sprouts	0.43 (0.22, 0.84) **0.01**	0.92 (0.79, 1.07) 0.28
Cabbage	0.29 (0.13, 0.63) **0.002**	0.94 (0.87, 1.02) 0.17
Capers	0.43 (0.20, 0.91) **0.03**	0.94 (0.84, 1.04) 0.21
Carrots	0.31 (0.10, 0.96) **0.04**	0.98 (0.94, 1.03) 0.42
Cauliflower	0.38 (0.16, 0.87) **0.02**	0.96 (0.88, 1.06) 0.44
Charcoal grilled veg	0.50 (0.06, 4.27) 0.52	0.73 (0.54, 1.00) **0.05**
Leeks	0.39 (0.19, 0.81) **0.01**	0.96 (0.83, 1.11) 0.60
Onions	0.32 (0.12, 0.86) **0.02**	0.90 (0.82, 0.98) **0.02**
Green salad	0.56 (0.19, 1.72) 0.32	0.96 (0.92, 1.00) 0.10
Parsnips	0.37 (0.18, 0.75) **0.01**	0.84 (0.61, 1.15) 0.28
Spinach	0.24 (0.09, 0.61) **0.003**	0.91 (0.80, 1.04) 0.16
Sweetcorn	0.32 (0.14, 0.73) **0.01**	0.93 (0.84, 1.02) 0.12
Watercress	0.37 (0.21, 0.67) **0.001**	0.74 (0.53, 1.04) 0.09

Dairy products		
Single cream	0.76 (0.35, 1.67) 0.50	0.99 (0.82, 1.17) 0.83
Clotted cream	0.64 (0.32, 1.26) 0.20	0.98 (0.81, 1.19) 0.88
Low fat yoghurt	0.70 (0.30, 1.68) 0.43	0.95 (0.87, 1.04) 0.27
Full fat yoghurt	0.74 (0.31, 1.76) 0.49	0.91 (0.80, 1.04) 0.18
Desserts	0.59 (0.28, 1.23) 0.16	0.89 (0.73, 1.07) 0.22
Cheese	0.93 (0.17, 5.02) 0.93	0.90 (0.84, 0.98) **0.01**
Eggs	0.38 (0.11, 1.31) 0.13	0.89 (0.79, 0.99) **0.04**
Quiche	0.74 (0.43, 1.29) 0.29	0.93 (0.74, 1.16) 0.51
Salad cream	0.43 (0.21, 0.90) **0.02**	0.98 (0.90, 1.06) 0.56
French dressing	0.53 (0.28, 1.02) 0.06	1.00 (0.94, 1.06) 0.95
Butter	0.37 (0.15, 0.92) **0.03**	1.00 (0.94, 1.07) 0.89
Margarine	0.44 (0.20, 0.99) **0.05**	1.02 (0.96, 1.09) 0.45
Low fat spread	0.51 (0.23, 1.13) 0.10	1.00 (0.93, 1.07) 0.98
Very low fat spread	0.50 (0.24, 1.04) 0.06	0.98 (0.90, 1.05) 0.52
Red Meat		
Beef	2.96 (1.50, 5.83) **0.002**	1.00 (0.91, 1.10) 0.93
Beef burgers	3.04 (1.69, 5.47) **<0.001**	1.00 (0.94, 1.05) 0.92
Bacon	3.91 (2.23, 6.84) **<0.001**	0.99 (0.88, 1.10) 0.79
Pork	1.97 (0.94, 4.15) 0.07	0.99 (0.92, 1.06) 0.75
Lamb	3.73 (2.12, 6.56) **<0.001**	0.94 (0.80, 1.09) 0.39
Ham	3.25 (1.83, 5.77) **<0.001**	0.94 (0.84, 1.06) 0.32
Corned beef	5.48 (3.21, 9.34) **<0.001**	0.88 (0.67, 1.16) 0.37
Sausages	5.00 (2.76, 9.02) **<0.001**	1.01 (0.93, 1.11) 0.77
Souvlaki	7.40 (1.00, 54.84) **0.05**	0.80 (0.62, 1.04) 0.09
Souvla	2.02 (0.24, 17.04) 0.52	0.49 (0.27, 0.87) **0.02**
Charcoal grilled meat	0.80 (0.78, 8.28) 0.85	0.77 (0.57, 1.04) 0.09
Grilled roasted meat	0.92 (0.36, 2.33) 0.86	0.93 (0.81, 1.07) 0.31
Other foods		
Beansprouts	0.31 (0.15, 0.61) **0.001**	0.93 (0.83, 1.04) 0.18
Coleslaw	0.34 (0.17, 0.70) **0.003**	0.95 (0.87, 1.03) 0.21

Garlic	0.35 (0.16, 0.78) **0.01**	0.91 (0.81, 1.01) 0.08
Dried pulses	0.36 (0.18, 0.70) **0.003**	0.84 (0.72, 1.00) **0.04**
Baked beans	0.38 (0.15, 0.94) **0.04**	0.92 (0.80, 1.07) 0.27
Mushrooms	0.45 (0.17, 1.23) 0.12	0.88 (0.77, 1.00) **0.05**
Veg. derived Protein		
Tofu	0.54 (0.29, 0.98) **0.04**	0.52 (0.24, 1.13) 0.10
White Meat		
Chicken	2.16 (0.88, 5.30) 0.09	0.98 (0.94, 1.03) 0.49
Cereal		
White bread	1.00 (0.38, 2.64) 0.99	0.96 (0.93, 1.00) 0.06
Whole meal Bread	0.61 (0.28, 1.37) 0.23	0.99 (0.95, 1.03) 0.58
Cream Crackers	0.72 (0.36, 1.41) 0.33	1.01 (0.95, 1.07) 0.73
Crispbread	0.94 (0.41, 2.19) 0.89	1.00 (0.95, 1.05) 0.96
Oat based cereal	0.46 (0.22, 0.94) **0.04**	0.94 (0.87, 1.00) 0.07
Refined breakfast cereal	0.31 (0.16, 0.60) **0.001**	0.90 (0.82, 1.00) **0.04**
Carbohydrates		
Potatoes	0.25 (0.09, 0.73) **0.01**	0.97 (0.89, 1.06) 0.49
Chips	1.08 (0.43, 2.70) 0.87	1.00 (0.93, 1.08) 0.95
Roast potatoes	0.83 (0.24, 2.83) 0.76	0.94 (0.82, 1.08) 0.39
Potato salad	0.71 (0.28, 1.76) 0.46	0.85 (0.65, 1.10) 0.21
White rice	1.32 (0.30, 5.83) 0.72	0.95 (0.86, 1.05) 0.29
Brown rice	0.54 (0.28, 1.06) 0.07	0.93 (0.79, 1.09) 0.37
White green pasta	0.34 (0.14, 0.85) **0.02**	0.94 (0.84, 1.04) 0.24
Whole meal pasta	0.45 (0.22, 0.93) **0.03**	0.83 (0.68, 1.02) 0.07
Lasagne	0.54 (0.24, 1.20) 0.13	0.90 (0.71, 1.15) 0.39
Pizza	0.55 (0.20, 1.50) 0.24	0.89 (0.70, 1.12) 0.30

Table 3 The association of individual foods and the likelihood of being consumed or not by cases, adjusted for sex. Determined by binary logistic regression. Dose response adjusted for sex

Food	**Binary (ate this fruit or not) adjusted for sex OR [95% C I] p**	**Dose response adjusted for sex OR [95% C I] p**

Fruit		
Apples	0.38 (0.14, 1.01) **0.05**	1.00 (0.97, 1.03) 0.93
Avocado	0.30 (0.16, 0.56) **<0.001**	0.96 (0.89, 1.03) 0.25
Bananas	0.52 (0.22, 1.25) 0.14	1.02 (0.99, 1.04) 0.26
Grapefruit	0.35 (0.18, 0.65) **0.001**	0.88 (0.78, 0.99) **0.03**
Grapes	0.32 (0.15, 0.68) **0.003**	0.97 (0.91, 1.02) 0.21
Marrow	0.55 (0.22, 1.40) 0.21	1.00 (0.97, 1.04) 0.83
Melon	0.46 (0.23, 0.95) **0.04**	1.00 (0.97, 1.05) 0.74
Oranges	0.49 (0.21, 1.13) 0.09	1.03 (1.00, 1.05) 0.06
Peaches	0.35 (0.17, 0.71) **0.004**	1.00 (0.95, 1.05) 0.65
Pears	0.30 (0.14, 0.67) **0.003**	0.97 (0.92, 1.02) 0.19
Peas	0.54 (0.24, 1.22) 0.14	0.84 (0.71, 0.99) **0.04**
Strawberries	0.40 (0.19, 0.83) **0.01**	1.00 (0.96, 1.03) 0.86
Sweet peppers	0.27 (0.13, 0.56) **<0.001**	0.90 (0.81, 0.99) **0.03**
Tomatoes	0.41 (0.18, 0.92) **0.03**	0.99 (0.96, 1.02) **<0.001**
Dried fruit	0.34 (0.17, 0.68) **0.002**	0.99 (0.96, 1.03) 0.76
Tinned fruit	0.27 (0.15, 0.46) **<0.001**	0.81 (0.65, 1.01) 0.06
Fish		
Fried fish in batter	3.53 (2.12, 5.88) **<0.001**	0.95 (0.86, 1.06) 0.39
Fish fingers	2.99 (1.87, 4.78) **<0.001**	0.91 (0.78, 1.07) 0.24
White fish	2.62 (1.55, 4.43) **<0.001**	0.97 (0.88, 1.06) 0.46
Oily fish	3.50 (1.88, 6.49) **<0.001**	0.98 (0.91, 1.06) 0.67
Shell fish	2.94 (1.77, 4.91) **<0.001**	0.98 (0.91, 1.04) 0.48
Fish roe	3.53 (2.21, 5.63) **<0.001**	1.00 (0.95, 1.06) 0.93
Vegetables		
Beetroot	0.36 (0.19, 0.68) **0.001**	0.81 (0.67, 0.97) **0.02**
Broccoli	0.30 (0.14, 0.62) **0.001**	0.92 (0.83, 1.01) 0.09
Brussels sprouts	0.27 (0.15, 0.49) **<0.001**	0.82 (0.68, 0.98) **0.03**
Cabbage	0.21 (0.11, 0.41) **<0.001**	0.93 (0.87, 1.01) 0.07
Capers	0.41 (0.22, 0.77) **0.01**	0.92 (0.83, 1.02) 0.11
Carrots	0.40 (0.15, 1.06) 0.07	0.98 (0.94, 1.02) 0.30

Cauliflower	0.39 (0.19, 0.82) **0.01**	0.93 (0.84, 1.03) 0.17
Charcoal grilled veg	1.05 (0.12, 8.77) 0.97	0.73 (0.55, 0.97) **0.03**
Leeks	0.28 (0.15, 0.52) **<0.001**	0.90 (0.74, 1.09) 0.26
Onions	0.43 (0.18, 0.99) **0.05**	0.95 (0.90, 1.01) 0.09
Green salad	0.55 (0.22, 1.41) 0.21	0.98 (0.95, 1.02) 0.37
Parsnips	0.25 (0.14, 0.47) **<0.001**	0.83 (0.64, 1.08) 0.16
Spinach	0.34 (0.15, 0.77) **0.01**	0.88 (0.78, 1.00) 0.06
Sweetcorn	0.21 (0.11, 0.43) **<0.001**	0.87 (0.77, 0.98) **0.02**
Watercress	0.29 (0.17, 0.48) **<0.001**	0.68 (0.46, 0.99) **0.05**
Dairy products		
Single cream	0.44 (0.22, 0.85) **0.02**	0.80 (0.61, 1.04) 0.10
Clotted cream	0.35 (0.20, 0.63) **<0.001**	0.76 (0.54, 1.06) 0.11
Low fat yoghurt	0.48 (0.23, 1.01) **0.05**	0.92 (0.84, 1.01) 0.06
Full fat yoghurt	0.47 (0.22, 1.00) **0.05**	0.92 (0.82, 1.03) 0.15
Desserts	0.32 (0.17, 0.60) **<0.001**	0.77 (0.62, 0.96) **0.02**
Cheese	1.08 (0.24, 4.88) 0.92	0.95 (0.90, 1.01) 0.13
Eggs	0.45 (0.16, 1.28) 0.14	0.85 (0.76, 0.95) **0.01**
Quiche	0.55 (0.34, 0.90) **0.02**	0.80 (0.61, 1.03) 0.08
Salad cream	0.27 (0.15, 0.51) **<0.001**	0.93 (0.85, 1.02) 0.13
French dressing	0.28 (0.16, 0.49) **<0.001**	0.97 (0.92, 1.04) 0.35
Butter	0.23 (0.11, 0.50) **<0.001**	0.98 (0.92, 1.04) 0.45
Margarine	0.28 (0.14, 0.55) **<0.001**	1.01 (0.95, 1.06) 0.85
Low fat spread	0.33 (0.17, 0.64) **0.001**	0.98 (0.92, 1.04) 0.47
Very low fat spread	0.31 (0.17, 0.58) **<0.001**	0.94 (0.86, 1.03) 0.16
Red Meat		
Beef	1.79 (0.97, 3.28) 0.06	0.92 (0.83, 1.01) 0.09
Beef burgers	2.14 (1.25, 3.67) **0.01**	0.95 (0.88, 1.03) 0.20
Bacon	2.84 (1.72, 4.70) **<0.001**	0.92 (0.81, 1.05) 0.22
Pork	1.64 (0.83, 3.26) 0.16	0.94 (0.86, 1.02) 0.14
Lamb	3.08 (1.83, 5.20) **<0.001**	3.08 (1.83, 5.20) **<0.001**
Ham	2.38 (1.42, 3.97) **0.001**	0.89 (0.79, 1.00) **0.05**

Corned beef	4.26 (2.65, 6.85) **<0.001**	0.76 (0.58, 1.00) **0.05**
Sausages	4.52 (2.61, 7.80) **<0.001**	0.97 (0.89, 1.07) 0.60
Souvlaki	10.40 (1.43, 75.88) **0.02**	0.68 (0.53, 0.88) **0.003**
Souvla	1.94 (0.25, 15.15) 0.53	0.38 (0.20, 0.70) **0.002**
Charcoal grilled meat	1.06 (0.13, 8.86) 0.96	0.65 (0.48, 0.89) **0.007**
Grilled roasted meat	1.47 (0.62, 3.51) 0.39	0.83 (0.73, 0.93) **0.002**
Other foods		
Beansprouts	0.24 (0.13, 0.43) **<0.001**	0.92 (0.82, 1.04) 0.17
Coleslaw	0.29 (0.15, 0.54) **<0.001**	0.97 (0.90, 1.03) 0.33
Garlic	0.29 (0.15, 0.56) **<0.001**	0.92 (0.83, 1.01) 0.08
Dried pulses	0.23 (0.13, 0.41) **<0.001**	0.83 (0.70, 0.98) **0.03**
Baked beans	0.47 (0.22, 1.04) 0.06	0.93 (0.83, 1.04) 0.18
Mushrooms	0.38 (0.16, 0.90) **0.03**	0.85 (0.75, 0.96) **0.01**
Veg. derived Protein		
Tofu	0.29 (0.17, 0.49) **<0.001**	0.27 (0.09, 0.82) **0.02**
White Meat		
Chicken	2.27 (0.18, 5.34) **0.06**	0.95 (0.90, 1.00) 0.06
Cereal		
White bread	0.43 (0.18, 0.99) **0.05**	0.99 (0.95, 1.02) 0.45
Whole meal Bread	0.34 (0.17, 0.69) **0.002**	0.99 (0.96, 1.02) 0.55
Cream Crackers	0.35 (0.19, 0.63) **0.001**	0.99 (0.93, 1.05) 0.73
Crispbread	0.45 (0.22, 0.92) **0.03**	1.00 (0.95, 1.05) 0.97
Oat based cereal	0.32 (0.17, 0.61) **<0.001**	0.93 (0.86, 0.99) **0.03**
Refined breakfast cereal	0.19 (0.11, 0.34) **<0.001**	0.85 (0.76, 0.94) **0.001**
Carbohydrates		
Potatoes	0.37 (0.15, 0.91) **0.03**	0.93 (0.85, 1.02) 0.13
Chips	0.50 (0.23, 1.09) 0.08	0.93 (0.84, 1.03) 0.16
Roast potatoes	0.80 (0.27, 2.40) 0.69	0.87 (0.75, 1.01) 0.07
Potato salad	0.49 (0.23, 1.08) 0.08	0.77 (0.60, 0.99) **0.04**
White rice	0.97 (0.28, 3.38) 0.96	0.87 (0.77, 0.99) **0.03**
Brown rice	0.36 (0.20, 0.63) **<0.001**	0.79 (0.63, 0.98) **0.03**

White green pasta	0.30 (0.14, 0.65) **0.002**	0.86 (0.75, 0.98) **0.03**
Whole meal pasta	0.39 (0.21, 0.74) **0.004**	0.74 (0.59, 0.91) **0.005**
Lasagne	0.31 (0.16, 0.62) **0.001**	0.72 (0.53, 0.98) **0.04**
Pizza	0.38 (0.16, 0.90) **0.03**	0.69 (0.51, 0.94) **0.02**

Table 4 The association of individual foods and the likelihood of being consumed or not by cases, adjusted for age and sex. Determined by binary logistic regression. Dose response adjusted for age and sex.

Food	**Binary (ate this fruit or not) OR [95% C I] Adj for age and sex P**	**Dose response OR [95% C I] Adj for age and sex P**
Fruit		
Apples	0.21 (0.07, 0.64) **0.01**	0.99 (0.96, 1.03) 0.71
Avocado	0.37 (0.18, 0.74) **0.01**	0.97 (0.90, 1.06) 0.52
Bananas	0.53 (0.19, 1.52) 0.24	1.03 (1.00, 1.06) 0.08
Grapefruit	0.46 (0.22, 0.96) **0.04**	0.87 (0.77, 1.00) **0.05**
Grapes	0.34 (0.14, 0.84) **0.02**	0.96 (0.90, 1.02) 0.15
Marrow	0.32 (0.10, 0.98) **0.05**	1.00 (0.95, 1.04) 0.82
Melon	0.39 (0.17, 0.88) 0.02	1.00 (0.95, 1.04) 0.84
Oranges	0.34 (0.14, 0.85) **0.02**	1.00 (0.97, 1.04) 0.75
Peaches	0.35 (0.15, 0.82) **0.02**	0.99 (0.95, 1.04) 0.78
Pears	0.24 (0.09, 0.62) **0.003**	0.97 (0.91, 1.02) 0.23
Peas	0.45 (0.17, 1.21) 0.11	0.92 (0.79, 1.06) 0.25
Strawberries	0.32 (0.14, 0.75) **0.01**	1.01 (0.97, 1.05) 0.71
Sweet peppers	0.28 (0.12, 0.67) **0.004**	0.91 (0.82, 1.00) 0.06
Tomatoes	0.29 (0.12, 0.73) **0.01**	0.96 (0.92, 1.00) **0.05**
Dried fruit	0.48 (0.21, 1.10) 0.08	0.99 (0.95, 1.04) 0.78
Tinned fruit	0.42 (0.22, 0.80) **0.01**	0.85 (0.68, 1.06) 0.15
Fish		
Fried fish in batter	5.3 (2.99, 9.52) **<0.001**	1.01 (0.93, 1.10) 0.76

Fish fingers	3.98 (2.35, 6.73) **<0.001**	0.99 (0.89, 1.10) 0.85
White fish	2.95 (1.66, 5.24) **<0.001**	1.02 (0.94, 1.10) 0.66
Oily fish	3.77 (1.95, 7.31) **<0.001**	1.01 (0.94, 1.09) 0.74
Shell fish	3.98 (2.25, 7.04) **<0.001**	1.01 (0.95, 1.07) 0.76
Fish roe	4.30 (2.56, 7.21) **<0.001**	1.04 (0.98, 1.09) 0.23
Vegetables		
Beetroot	0.97 (0.62, 1.51) 0.89	0.75 (0.59, 0.95) **0.02**
Broccoli	0.42 (0.18, 1.00) **0.05**	0.97 (0.90, 1.05) 0.40
Brussels sprouts	0.96 (0.62, 1.50) 0.87	0.92 (0.79, 1.07) 0.29
Cabbage	0.29 (0.13, 0.64) **0.002**	0.94 (0.87, 1.03) 0.17
Capers	0.43 (0.21, 0.92) **0.03**	0.94 (0.84, 1.04) 0.22
Carrots	0.31 (0.10, 0.96) **0.04**	0.98 (0.94, 1.03) 0.43
Cauliflower	0.38 (0.17, 0.90) **0.02**	0.97 (0.88, 1.06) 0.46
Charcoal grilled veg	0.37 (0.18, 0.76) **0.01**	0.73 (0.53, 1.00) **0.05**
Leeks	0.40 (0.19, 0.82) **0.01**	0.96 (0.84, 1.11) 0.62
Onions	0.32 (0.12, 0.86) **0.02**	0.90 (0.82, 0.98) **0.02**
Green salad	0.56 (0.18, 1.72) 0.31	0.96 (0.92, 1.01) 0.10
Parsnips	0.37 (0.18, 0.76) **0.01**	0.84 (0.61, 1.16) 0.29
Spinach	0.24 (0.09, 0.62) **0.003**	0.91(0.80, 1.04) 0.16
Sweetcorn	0.32 (0.14, 0.74) **0.01**	0.93 (0.84, 1.02) 0.12
Watercress	0.38 (0.21, 0.68) **0.001**	0.75 (0.53, 1.05) 0.09
Dairy products		
Single cream	0.77 (0.35, 1.69) 0.51	0.98 (0.82, 1.17) 0.85
Clotted cream	0.65 (0.33, 1.28) 0.21	0.99 (0.82, 1.20) 0.90
Low fat yoghurt	0.71 (0.30, 1.69) 0.44	0.95 (0.87, 1.04) 0.25
Full fat yoghurt	0.93 (0.60, 1.44) 0.73	0.91 (0.80, 1.04) 0.18
Desserts	0.59 (0.28, 1.25) 0.17	0.89 (0.73, 1.07) 0.22
Cheese	0.91 (0.17, 4.93) 0.91	0.90 (0.84, 0.98) **0.01**
Eggs	0.38 (0.11, 1.32) 0.13	0.88 (0.79, 0.99) **0.04**
Quiche	0.75 (0.44, 1.29) 0.30	0.93 (0.74, 1.16) 0.52
Salad cream	0.43 (0.21, 0.90) **0.03**	0.98 (0.90, 1.06) 0.56

French dressing	0.54 (0.28, 1.03) 0.06	0.98 (0.90, 1.06) 0.56
Butter	0.37 (0.15, 0.92) **0.03**	1.01 (0.94, 1.07) 0.89
Margarine	0.45 (0.20, 1.00) **0.05**	1.01 (0.94, 1.07) 0.89
Low fat spread	0.52 (0.24, 1.14) 0.10	1.02 (0.96, 1.09) 0.45
Very low fat spread	0.51 (0.25, 1.06) 0.07	0.98 (0.90, 1.05) 0.52
Red Meat		
Beef	3.00 (1.52, 5.93) **0.002**	1.00 (0.92, 1.10) 0.93
Beef burgers	3.06 (1.70, 5.51) **<0.001**	1.00 (0.94, 1.06) 0.93
Bacon	4.00 (2.26, 6.92) **<0.001**	0.99 (0.88, 1.10) 0.80
Pork	1.99 (0.95, 4.19) 0.07	0.99 (0.92, 1.06) 0.76
Lamb	3.78 (2.15, 6.65) **<0.001**	0.94 (0.81, 1.09) 0.39
Ham	3.27 (1.84, 5.80) **<0.001**	0.94 (0.84, 1.06) 0.33
Corned beef	5.52 (3.23, 9.41) **0.001**	0.88 (0.67, 1.16) 0.37
Sausages	5.07 (2.80, 9.17) **<0.001**	1.01(0.93, 1.11) 0.75
Souvlaki	7.37 (1.00, 54.56) **0.05**	0.80 (0.62, 1.04) 0.09
Souvla	2.01 (0.24, 16.90) 0.52	0.48 (0.27, 0.87) 0.02
Charcoal grilled meat	0.82 (0.08, 8.54) 0.87	0.77 (0.56, 1.04) 0.09
Grilled roasted meat	0.92 (0.36, 2.33) 0.86	0.93 (0.81, 1.07) 0.31
Other foods		
Beansprouts	0.31 (0.16, 0.62) **0.001**	0.93 (0.83, 1.04) 0.18
Coleslaw	0.34 (0.17, 0.70) **0.004**	0.95 (0.88, 1.03) 0.22
Garlic	0.35 (0.16, 0.79**)** **0.01**	0.91(0.81, 1.01) 0.08
Dried pulses	0.36 (0.18, 0.70) **0.003**	0.84 (0.72, 0.99) **0.04**
Mushrooms	0.45 (0.17, 1.22) 0.11	0.88 (0.77, 1.00) **0.05**
Veg. derived Protein		
Tofu	0.53 (0.29, 0.97) **0.04**	0.53 (0.24, 1.14) 0.10
White Meat		
Chicken	2.14 (0.87, 5.27) 0.10	0.98 (0.94, 1.03) 0.48
Cereal		
White bread	1.00 (0.38, 2.65) 1.00	0.96 (0.93, 1.00) 0.06
Whole meal Bread	0.62 (0.28, 1.38) 0.24	0.99 (0.95, 1.03) 0.57

Cream Crackers	0.71 (0.36, 1.40) 0.33	1.01 (0.95, 1.07) 0.72
Crispbread	0.95 (0.41, 2.20) 0.90	1.00 (0.95, 1.06) 0.93
Oat based cereal	0.45 (0.22, 0.95) **0.04**	0.94 (0.87, 1.01) 0.07
Refined breakfast cereal	0.31 (0.16, 0.61) **0.001**	0.90 (0.82, 0.99) **0.04**
Carbohydrates		
Potatoes	0.25 (0.09, 0.74) **0.01**	0.97 (0.89, 1.06) 0.50
Chips	1.09 (0.43, 2.72) 0.86	1.00 (0.93, 1.08) 0.92
Roast potatoes	0.82 (0.24, 2.82) 0.76	0.94 (0.83, 1.08) 0.40
Potato salad	0.71(0.29, 1.78) 0.47	0.85 (0.65, 1.10) 0.22
White rice	1.28 (0.29, 5.67) 0.75	0.95 (0.86, 1.05) 0.29
Brown rice	0.55 (0.28, 1.07) 0.08	0.93 (0.80, 1.09) 0.37
White green pasta	0.35 (0.14, 0.86) **0.02**	0.94 (0.84, 1.04) 0.24
Whole meal pasta	0.46 (0.22, 0.94) **0.03**	0.83 (0.68, 1.02) 0.07
Lasagne	0.55 (0.25, 1.22) 0.14	0.90 (0.71, 1.15) 0.40
Pizza	0.55 (0.20, 1.52) 0.25	0.89 (0.71, 1.11) 0.31

Table 5 Age-matched analysis Conditional Logistic Regression unadjusted and adjusted for sex of individual foods together with dose response

	Unadjusted for sex		**Adjusted for sex**	
Food	**Odds Ratio (95% CI) p**	**Dose response Odds Ratio**	**Odds Ratio (95% CI) p**	**Dose response Odds Ratio [95% C I] p**
Fruit				
Apples	0.33 (0.07, 1.65) 0.18	0.99 (0.94, 1.04) 0.65	0.30 (0.06, 1.53) 0.15	0.99 (0.95, 1.04) 0.69
Avocado	0.25 (0.08, 0.75) **0.01**	0.92 (0.81, 1.05) 0.23	0.26 (0.09, 0.76) **0.02**	0.92 (0.81, 1.05) 0.21
Bananas	1.63e-18 0.00 1.00	1.02 (0.97, 1.07) 0.41	1.75e-07 0.00 0.99	1.02 (0.97, 1.07) 0.44
Grapefruit	0.21 (0.06, 0.75) **0.02**	0.79 (0.66, 0.94) **0.01**	0.20 (0.06, 0.71) **0.01**	0.79 (0.66, 0.95) **0.01**
Grapes	0.20 (0.04, 0.91) **0.04**	0.95 (0.88, 1.02) 0.15	0.19 (0.04, 0.88) **0.03**	0.95 (0.88, 1.02) 0.18
Marrow	0.40 (0.08, 2.06) 0.27	1.02 (0.96, 1.08) 0.50	0.38 (0.07, 1.98) 0.25	1.02 (0.96, 1.08) 0.60
Melon	0.20 (0 .04, 0.91) **0.04**	0.99 (0.93, 1.04) 0.65	0.19 (0.04, 0.88) **0.03**	0.99 (0.94, 1.05) 0.70
Oranges	0.25 (0.05, 1.18) 0.08	1.01 (0.97, 1.06) 0.58	0.24 (0.05, 1.14) 0.07	1.02 (0 .97, 1.07) 0.55
Peaches	0.27 (0.08, 0.98) **0.05**	0.97 (0.92, 1.03) 0.34	0.28 (0.08, 1.01) **0.05**	0.98 (0.93, 1.03) 0.37

Pears	0.11 (0.01, 0.88) **0.04**	0.96 (0.89, 1.02) 0.19	0.10 (0.01, 0.81) **0.03**	0.96 (0.90, 1.02) 0.21
Peas	0.14 (0.02, 1.16) 0.07	0.86 (0.69, 1.07) 0.18	0.12 (0.02, 1.01) **0.05**	0.85 (0.67, 1.08) 0.17
Strawberries	0.18 (0.04, 0.82) **0.03**	0.99 (0.95, 1.04) 0.81	0 .18 (0.04, 0.81) **0.03**	1.00 (0.95, 1.04) 0.85
Sweet peppers	1.22e-17 0.00 1.00	0.88 (0.77, 1.01) 0.07	9.17e-08 0.0 0.99	0.88 (0.76, 1.01) 0.06
Tomatoes	0.25 (0.05, 1.18) 0.08	0.95 (0.91, 1.00) **0.05**	0.25 (0.05, 1.17) 0.08	0.95 (0.91, 1.00) **0.05**
Dried fruit	0.11 (0.01, 0.88) **0.04**	0.97 (0.92, 1.02) 0.19	0.11 (0.01, 0.90) **0.04**	0.97 (0.92, 1.02) 0.23
Tinned fruit	0.32 (0.13, 0.79) **0.01**	0.87 (0.70, 1.08) 0.20	0.31 (0.12, 0.78) **0.01**	0.86 (0.69, 1.08) 0.21
Fish				
Fried fish in batter	6.43 (2.90, 14.26) **<0.001**	1.02 (0.87, 1.21) 0.79	6.54 (2.94, 14.57) **<0.001**	1.02 (0.86, 1.20) 0.84
Fish fingers	3.90 (1.95, 7.81) **<0.001**	1.10 (0.82, 1.46) 0.53	3.89 (1.94, 7.80) **<0.001**	1.11 (0.83, 1.49) 0.47
White fish	2.20 (1.04, 4.65) **0.04**	0.99 (0.86, 1.14) 0.88	2.24 (1.06, 4.74) **0.04**	0.99 (0.85, 1.14) 0.86
Oily fish	3.83 (1.56, 9.41) **0.003**	0.97 (0.86, 1.10) 0.66	3.96 (1.61, 9.78) **0.003**	0.97 (0.86, 1.10) 0.64
Shell fish	3.88 (1.78, 8.43) **0.001**	0.97 (0.90, 1.05) 0.43	3.96 (1.81, 8.65) **0.001**	0.97 (0.90, 1.04) 0.36
Fish roe	5.00 (2.34, 10.68) **<0.001**	1.04 (0.93, 1.17) 0.45	4.93 (2.3, 10.55) **<0.001**	1.04 (0.94, 1.16) 0.45
Vegetables				
Beetroot	0.15 (0.04, 0.68) **0.01**	0.71 (0.55, 0.91) **0.01**	0.16 (0.04, 0.71) **0.02**	0.71 (0.55, 0.91) **0.01**
Broccoli	0.30 (0.08, 1.09) 0.07	0.93 (0.83, 1.05) 0.24	0.30 (0.08, 1.09) 0.07	0.93 (0.83, 1.05) 0.24
Brussels sprouts	0.29 (0.11, 0.80) **0.02**	0.84 (0.68, 1.04) 0.11	0.30 (0.11, 0.82) **0.02**	0.84 (0.67, 1.05) 0.12
Cabbage	0.14 (0.03, 0.63) **0.01**	0.90 (0.79, 1.02) 0.11	0.15 (0.03, 0.64) **0.01**	0.90 (0.79, 1.03) 0.13
Capers	0.31 (0.10, 0.94) **0.04**	0.91 (0.80, 1.04) 0.15	0.31 (0.10, 0.96) **0.04**	0.91 (0.80, 1.04) 0.16
Carrots	0.20 (0.02, 1.71) 0.14	0.97 (0.92, 1.03) 0.31	0.19 (0.02, 1.63) 0.13	0.97 (0.91, 1.03) 0.27
Cauliflower	0.22 (0.05, 1.03) **0.05**	0.99 (0.83, 1.18) 0.88	0.23 (0.05, 1.09) 0.06	0.98 (0.81, 1.17) 0.79
Charcoal grilled veg	8.10e-16 0.00 1.00	0.65 (0.41, 1.05) 0.08	4.26e-07 0.00 0.99	0.68 (0.43, 1.07) 0.09
Leeks	0.27 (0.09, 0.80) **0.02**	1.10 (0.77, 1.59) 0.60	0.27 (0.09, 0.81) **0.02**	1.09 (0.75, 1.57) 0.66
Onions	8.10e-16 0.00 1.00	0.89 (0.80, 0.98) **0.02**	7.51e-08 0.00 0.99	0.89 (0.80, 0.98) **0.02**
Green salad	0.50 (0.09, 2.73) 0.42	0.96 (0.91, 1.01) 0.12	0.42 (0.08, 2.38) 0.33	0.96 (0.91, 1.01) 0.10
Parsnips	0.19 (0.06, 0.64) **0.01**	0.84 (0.60, 1.20) 0.34	0.19 (0.06, 0.66) **0.01**	0.85 (0.60, 1.21) 0.37
Spinach	0.11 (0.01, 0.88) **0.04**	0.81 (0.65, 1.01) 0.06	0.11 (0.01, 0.84) **0.03**	0.81 (0.65, 1.01) 0.06
Sweetcorn	0.08 (0.01, 0.64) **0.02**	0.93 (0.83, 1.03) 0.17	0.08 (0.01, 0.65) **0.02**	0.93 (0.83, 1.04) 0.18
Watercress	0.19 (0.07, 0.56) **0.002**	0.68 (0.44, 1.06) 0.09	0.19 (0.07, 0.57) **0.003**	0.69 (0.45, 1.07) 0.10
Dairy products				

Single cream	0.21 (0.06, 0.75) **0.02**	0.97 (0.71, 1.32) 0.84	0.45 (0.14, 1.47) 0.19	0.99 (0.72, 1.35) 0.92
Clotted cream	0.50 (0.20, 1.23) 0.13	0.95 (0.68, 1.33) 0.75	0.50 (0.20, 1.25) 0.14	0.95 (0.68, 1.34) 0.78
Low fat yoghurt	0.88 (0.32, 2.41) 0.80	0.96 (0.89, 1.03) 0.24	0.81 (0.29, 2.26) 0.69	0.95 (0.88, 1.03) 0.19
Full fat yoghurt	0.75 (0.26, 2.16) 0.60	0.94 (0.82, 1.07) 0.34	0.78 (0.27, 2.25) 0.64	0.93 (0.81, 1.06) 0.27
Desserts	0.31 (0.10, 0.94) **0.04**	0.94 (0.80, 1.09) 0.40	0.29 (0.10, 0.91) **0.03**	0.92 (0.79, 1.08) 0.33
Cheese	1.00 (0.14, 7.10) 1.00	0.91 (0.84, 0.99) **0.04**	0.87 (0.12, 6.32) 0.89	0.90 (0.82, 0.98) **0.02**
Eggs	0.40 (0.08, 2.06) 0.27	0.88 (0.76, 1.02) 0.08	0.40 (0.08, 2.06) 0.27	0.86 (0.74, 1.00) **0.05**
Quiche	0.74 (0.37, 1.47) 0.39	0.89 (0.67, 1.17) 0.40	0.75 (0.38, 1.51) 0.42	0.89 (0.67, 1.17) 0.40
Salad cream	0.20 (0.06, 0.69) **0.01**	0.98 (0.90, 1.07) 0.71	0.20 (0.06, 0.68) **0.01**	0.98 (0.90, 1.07) 0.70
French dressing	0.38 (0.16, 0.93) **0.03**	1.03 (0.91, 1.17) 0.59	0.40 (0.17, 0.96) **0.04**	1.04 (0.92, 1.17) 0.51
Butter	0.10 (0.01, 0.78) **0.03**	1.06 (0.94, 1.20) 0.31	0.09 (0.01, 0.73) **0.02**	1.06 (0.94, 1.19) 0.38
Margarine	0.18 (0.04, 0.82) **0.03**	1.02 (0.94, 1.11) 0.58	0.18 (0.04, 0.80) **0.02**	1.02 (0.93, 1.11) 0.70
Low fat spread	0.58 (0.23, 1.48) 0.26	1.00 (0.92, 1.09) 0.94	0.58 (0.23, 1.48) 0.26	1.00 (0.91, 1.09) 0.92
Very low fat spread	0.39 (0.14, 1.08) 0.07	0.95 (0.86, 1.04) 0.27	0.39 (0.14, 1.11) 0.08	0.94 (0.85, 1.04) 0.20
Red Meat				
Beef	3.00 (1.28, 7.06) **0.01**	0.98(0.87, 1.11) 0.77	3.75 (1.68, 8.37) **0.001**	0.98 (0.87, 1.11) 0.79
Beef burgers	3.50 (1.60, 7.68) **0.002**	1.00 (0.92, 1.08) 0.98	3.75 (1.68, 8.37) **0.001**	1.00 (0.92, 1.08) 0.97
Bacon	4.63 (2.15,9.93) **<0.001**	1.06 (0.89, 1.25) 0.52	4.78 (2.21, 10.33) **<0.001**	1.07 (0.90, 1.27) 0.45
Pork	2.00 (0.90, 4.45) 0.09	0.99 (0.89, 1.09) 0.77	1.99 (0.89, 4.43) 0.09	0.98 (0.89, 1.08) 0.67
Lamb	3.20 (1.57,6.51) **0.001**	0.79 (0.61, 1.02) 0.07	3.49 (1.68, 7.24) **0.001**	0.80 (0.62, 1.04) 0.10
Ham	3.89 (1.87, 8.09) **<0.001**	0.97 (0.88, 1.07) 0.56	3.97 (1.90, 8.30) **<0.001**	0.97 (0.88, 1.08) 0.60
Corned beef	5.88 (2.78, 12.43) **<0.001**	1.07 (0.67, 1.70) 0.79	6.05 (2.84, 12.92) **<0.001**	1.09 (0.68, 1.73) 0.73
Sausages	5.86 (2.63, 13.06) **<0.001**	1.32 (0.79, 2.21) 0.29	6.16 (2.74, 13.88) **<0.001**	1.38 (0.81, 2.34) 0.23
Souvlaki	1.16e+17 0.00 1.00	0.80 (0.57, 1.13) 0.21	e 0.00 0.99	0.81 (0.58, 1.14) 0.23
Souvla	8.10e-16 0.00 1.00	0.61 (0.33, 1.14) 0.12	1.10e-09 0.00 1.00	0.61 (0.33, 1.13) 0.11
Charcoal grilled meat	8.10e-16 0.00 1.00	0.81 (0.56, 1.15) 0.24	4.26e-07 0.00 0.99	0.80 (0.56, 1.14) 0.21
Grilled roasted meat	1.00 (0.32, 3.10) 1.00	0.98 (0.81, 1.17) 0.79	0.93 (0.30, 2.93) 0.90	0.97 (0.81, 1.16) 0.73
Other foods				
Beansprouts	0.31 (0.12, 0.85) **0.02**	0.93 (0.81, 1.06) 0.28	0.32 (0.12, 0.89) **0.03**	0.93 (0.81, 1.07) 0.30
Coleslaw	5.74e-18 0.0 1.00	0.90 (0.75, 1.07) 0.23	9.55e-09 0.00 1.00	0.90 (0.75, 1.07) 0.23

Garlic	0.23 (0.07, 0.89) **0.03**	0.93 (0.82, 1.06) 0.30	0.26 (0.07, 0.93) **0.04**	0.94 (0.82, 1.07) 0.34
Dried pulses	0.22 (0.08, 0.66) **0.01**	0.83 (0.70, 0.98) **0.03**	0.22 (0.08, 0.66) **0.01**	0.83 (0.70, 0.98) **0.03**
Mushrooms	0.17 (0.02, 1.38) 0.10	0.86 (0.73, 1.02) 0.08	0.18 (0.02, 1.52) 0.12	0.85 (0.72, 1.01) 0.07
Veg. derived Protein				
Tofu	0.38 (0.15, 0.96) **0.04**	0.37 (0.13, 1.03) 0.06	0.38 (0.15, 0.98) **0.05**	0.37 (0.13, 1.05) 0.06
White Meat				
Chicken	2.33 (0.90, 6.07) 0.08	1.01 (0.93, 1.09) 0.88	2.26 (0.87, 5.91) 0.10	1.00 (0.92, 1.09) 0.99
Cereal				
White bread	0.86 (0.29, 2.55) 0.78	0.97 (0.29, 2.55) 0.10	0.86 (0.29, 2.56) 0.78	0.97 (0.93, 1.01) 0.10
Whole meal Bread	0.64 (0.25, 1.64) 0.35	0.99 (0.95, 1.04) 0.72	0.62 (0.24, 1.62) 0.33	0.99 (0.95, 1.04) 0.71
Cream Crackers	0.79 (0.36, 1.73) 0.55	1.01 (0.94, 1.08) 0.87	0.79 (0.36, 1.75) 0.57	1.01 (0.94, 1.08) 0.79
Crispbread	0.67 (0.24, 1.87) 0.44	0.98 (0.93, 1.03) 0.38	0.68 (0.24, 1.91) 0.46	0.98 (0.93, 1.03) 0.43
Oat based cereal	0.14 (0.03, 0.63) **0.01**	0.90 (0.81, 1.00) **0.04**	0.13 (0.03, 0.60) **0.01**	0.90 (0.82, 1.00) **0.05**
Refined breakfast cereal	0.15 (0.05, 0.51) **0.002**	0.88 (0.78, 0.99) **0.03**	0.15 (0.04, 0.49) **0.002**	0.88 (0.78, 0.99) **0.04**
Carbohydrates				
Potatoes	8.10e-16 0.00 1.00	0.97 (0.87, 1.08) 0.58	5.76e-08 0.00 0.99	0.98 (0.88, 1.09) 0.66
Chips	0.60 (0.14, 2.51) 0.48	1.03 (0.90, 1.17) 0.69	0.53 (0.13, 2.27) 0.39	1.03 (0.90, 1.17) 0.66
Roast potatoes	0.50 (0.09, 2.73) 0.42	1.03 (0.82, 1.29) 0.82	0.47 (0.09, 2.61) 0.39	1.03 (0.82, 1.30) 0.79
Potato salad	0.29 (0.06, 1.37) 0.12	0.85 (0.60, 1.21) 0.37	0.26 (0.05, 1.28) 0.10	0.84 (0.59, 1.18) 0.31
White rice	0.67 (0.11, 3.99) 0.66	0.95 (0.84, 1.07) 0.38	0.55 (0.09, 3.43) 0.52	0.94 (0.83, 1.06) 0.30
Brown rice	0.20 (0.06, 0.69) **0.01**	0.85 (0.66, 1.10) 0.22	0.20 (0.06, 0.71) **0.01**	0.85 (0.66, 1.10) 0.22
White green pasta	0.13 (0.02, 1.00) **0.05**	0.94 (0.76, 1.15) 0.54	0.10 (0.01, 0.83) **0.03**	0.93 (0.75, 1.14) 0.47
Whole meal pasta	0.36 (0.13, 0.99) **0.05**	0.79 (0.63, 1.00) **0.05**	0.37 (0.13, 1.03) 0.06	0.79 (0.62, 1.00) **0.05**
Lasagne	0.30 (0.08, 1.09) 0.07	0.93 (0.65, 1.32) 0.68	0.30 (0.08, 1.09) 0.07	0.92 (0.65, 1.31) 0.65
Pizza	0.29 (0.06, 1.38) 0.12	0.80 (0.57, 1.11) 0.18	0.25 (0.05, 1.24) 0.09	0.80 (0.58, 1.11) 0.18

Appendix C

The STROBE "checklist" has been reproduced here.

STROBE Statement—Checklist of items that should be included in reports of case-control studies (Source http://www.strobe-statement.org.2019).

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(*b*) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions

		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how matching of cases and controls was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

Appendix D 1 Consent Form in English

You are being asked to participate in a research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. This consent may contain words that you do not understand. Please ask Dr Johnson to explain any words or information that you do not clearly understand.

Purpose of Study

The study aims to establish which environmental factors are a risk of acquiring Multiple Sclerosis among Cypriots.

8.1 Study Procedures

Participation in this study will only be during the time when the questions are being asked. You can stop participating at any time.

8.2 ***Confidentiality***

8.3 *Information gathered in this research study may be published or presented in public forums, however, your name and other identifying information will not be used or revealed*

8.4 *Voluntary Participation/ Withdrawal from the Study*

You may refuse to answer individual questions.

8.5 ***Questions***

You are free to ask any questions that you may have about this research study and your rights as a research participant. If any questions come up during the study please ask Dr.Paul Johnson.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all your questions.

Legal Rights

You are not waiving any of your legal rights by signing this consent form nor releasing the investigator from their legal or professional responsibility.

8.5.1 ***Statement of Consent***

I have read the Participant Information Sheet and
this consent form. I have had the opportunity to discuss this research study with Dr.Paul Johnson. I have had my questions answered in a way I understand. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study. I understand that information regarding my personal identity will be kept confidential.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in this study.

I consent to the following as part of this study:

a) Filling in the research questionnaire *Yes* *No*

b) Having my data included in medical journal articles, conference presentations, etc. however my name and other identifying information will not be used or revealed. *Yes* *No*

_____

_____

Signature of Participant *Date*

Appendix D2 Consent Form in Greek

Έντυπο Συγκατάθεσης

Καλείστε να συμμετάσχετε σε μια ερευνητική μελέτη. Παρακαλώ όπως αφιερώσετε τον κατάλληλο χρόνο για να μελετήσετε το έντυπο συγκατάθεσης και να συζητήσετε τυχόν ερωτήσεις με το προσωπικό της μελέτης.
Αυτό το έντυπο συγκατάθεσης μπορεί να περιέχει λέξεις που δεν καταλαβαίνετε. Παρακαλώ ζητήστε από τον Δρ.Johnson να σας εξηγήσει οποιεσδήποτε λέξεις ή πληροφορίες δεν καταλαβαίνετε.

Σκοπός Μελέτης
Η μελέτη στοχεύει στο να διαπιστώσει ποιοι περιβαλλοντικοί παράγοντες αποτελούν κίνδυνο εμφάνισης σκλήρυνσης κατά πλάκας στους Κύπριους.

8.6 Διαδικασία Μελέτης
Η συμμετοχή στη μελέτη αυτή θα διαρκεί μόνο κατά την περίοδο που υποβάλλονται οι ερωτήσεις. Μπορείτε να σταματήσετε να συμμετέχετε ανά πάσα στιγμή.

8.7 Εμπιστευτικότητα
8.8 Οι πληροφορίες που θα συγκεντρωθούν στην μελέτη αυτή μπορούν να δημοσιευθούν ή να παρουσιαστούν σε δημόσια φόρουμ, ωστόσο το όνομά σας και άλλες πληροφορίες ταυτοποίησης δεν θα χρησιμοποιηθούν ούτε θα αποκαλυφθούν.

8.9 Εθελοντική Συμμετοχή / Απόσυρση από τη Μελέτη
Έχετε το δικαίωμα να αρνηθείτε να απαντήσετε σε μεμονωμένες ερωτήσεις.

8.10 Ερωτήσεις
Είστε ελεύθεροι να θέσετε οποιαδήποτε ερωτήματα έχετε σχετικά με τη μελέτη αυτή καθώς και για τα δικαιώματά σας ως συμμετέχοντας/συμμετέχουσα στην έρευνα. Εάν προκύψουν οποιαδήποτε ερωτήματα κατά τη διάρκεια της μελέτης, παρακαλώ όπως ρωτήστε τον Δρ.Paul Johnson.

Μην υπογράψετε αυτήν τη φόρμα συγκατάθεσης εάν δεν είχατε την ευκαιρία να υποβάλετε τυχόν ερωτήσεις και δεν έχετε λάβει ικανοποιητικές απαντήσεις σε αυτές.

Νόμιμα δικαιώματα
Υπογράφοντας αυτό το έντυπο συγκατάθεσης, δεν παραιτείστε από τα νόμιμα δικαιώματά σας, ούτε απαλλάσσετε τον ερευνητή από τη νομική ή επαγγελματική ευθύνη του.

Η μελέτη αυτή έχει εγκριθεί από την Επιτροπή Δεοντολογίας Έρευνας της Σχολής Ιατρικών Επιστημών, μέρος της Επιτροπής Δεοντολογίας Έρευνας του Newcastle University. Η επιτροπή αυτή περιλαμβάνει μέλη τα οποία είναι εσωτερικά της Σχολής, καθώς και ένα εξωτερικό μέλος. Η μελέτη αυτή εξετάστηκε από τα μέλη της επιτροπής, τα οποία πρέπει να παρέχουν αμερόληπτες συμβουλές και να αποφεύγουν σημαντικές συγκρούσεις συμφερόντων.

8.10.1 *Δήλωση συγκατάθεσης*
Έχω διαβάσει το Δελτίο Πληροφοριών Συμμετεχόντων και το παρόν Έντυπο συγκατάθεσης. Είχα την ευκαιρία να συζητήσω την παρούσα ερευνητική μελέτη με τον Δρ.Paul Johnson. Τα ερωτήματά μου έχουν απαντηθεί με κατανοητό τρόπο. Κατανοώ ότι

η συμμετοχή μου σε αυτή τη μελέτη είναι εθελοντική και ότι μπορώ να επιλέξω να αποσυρθώ ανά πάσα στιγμή. Συμφωνώ με ελεύθερη βούληση να συμμετάσχω σε αυτή τη μελέτη. Κατανοώ το ότι οι πληροφορίες σχετικά με την προσωπική μου ταυτότητα θα παραμείνουν εμπιστευτικές.

Ως μέρος αυτής της μελέτης, δηλώνω την συγκατάθεση μου ως προς τα πιο κάτω:

α) Συμπλήρωση του ερωτηματολογίου της έρευνας Ναι Όχι

β) Να συμπεριληφθούν τα δεδομένα μου σε άρθρα ιατρικών περιοδικών, παρουσιάσεις, συνέδρια κλπ., ωστόσο το όνομά μου και άλλες πληροφορίες ταυτοποίησης δεν θα χρησιμοποιηθούν ούτε θα αποκαλυφθούν.

Ναι Όχι

_____ _____

Υπογραφή συμμετέχοντα Ημερομηνία

_____

Ονοματεπώνυμο συμμετέχοντα

Appendix E 1 Participant Information Sheet in English

Study name
A Case-Control study of Multiple Sclerosis among in Cyprus

You are invited to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Ask questions if anything you read is not clear or would like more information. Take time to decide whether or not to take part.

This is a questionnaire based study.

What is the purpose of the study?

The study aims to establish which environmental factors are a risk of acquiring Multiple Sclerosis among Cypriots.

Why have I been invited?
You have been chosen because you are a Cypriot. This study is not for people of other nationalities.
Some people are chosen because they have Multiple Sclerosis. These are the cases.
Others are chosen because they don't have Multiple Sclerosis. These are the controls.
The environmental factors that the control group have been exposed to will be compared to those of the people with Multiple Sclerosis.

Do I have to take part?
No. Your participation in the research is entirely voluntary. If you have Multiple Sclerosis your current and future treatment will be unaltered whether or not, you take part. If you do not have Multiple Sclerosis any other medical treatment you receive now or need in the future will not be affected either.

What will happen to me if I take part?
You will be asked to fill in a questionnaire that will ask about food and lifestyle.

It will take between 10-15 minutes. You will only be asked to fill this in once and won't be contacted for any other information.

Expenses and payments?
There is no payment for this study.

What will I have to do?
Fill in a questionnaire.

What are the possible disadvantages and risks of taking part?
There are none.

What are the possible benefits of taking part?
There are no direct benefits available for you. However, your participation will help enrich our current understanding of the epidemiology, pathophysiology and burden of Multiple Sclerosis in the Cypriot population. This is something that will be extremely useful for devising targeted prevention programmes that will be more efficient and cost-effective, thus reducing the burden of the disease on the society and at the same time saving money from the National Health System of Cyprus.

What if there is a problem?
This is unlikely but if you have a concern about any aspect of this study, you should ask to speak to the researcher Dr Paul Johnson who will do his best to answer your questions or you may email: Johnson.p@unic.ac.cy

If you remain unhappy and wish to complain formally you can do this to Dr Alexandros Heraclides by email to: heraclides.a@unic.ac.cy

Will my taking part in the study be kept confidential?
All questionnaires will be anonymised, and participants will be identified by a specific study number. Only the principal investigator will have access to the patient's name and this information will be kept, password-protected, in a separate file in the principal investigator's computer only. No other person will be able to link participant names to any information collected from the questionnaire.

All electronic information (the database) of the research project will be securely stored in a password-protected electronic file. Only the principal investigator will be aware of the password, which will be changed every 3 months.

Completed questionnaires will be locked in a safe locker in the study coordinator's office at CING immediately after data collection. Once the transfer from the paper questionnaires to the electronic dataset is completed, all questionnaires will be safely stored in a locked metallic drawer. No person other than the study principal investigator will have access to these questionnaires. All computers containing study data, will be safely locked at the CING premises when not in operation.

All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves the hospital/surgery/university will have your name and address removed so that you cannot be recognised.

What will happen to the results of the research study?
Any published material will be anonymized so you cannot be identified.

Who is organising or sponsoring the research?
This research is organised by Dr Paul Johnson the principal investigator. There is no funding or sponsorship.

Appendix E 2 Participant Information Sheet in Greek

Δελτίο Πληροφοριών Συμμετεχόντων
Όνομα μελέτης
Μια μελέτη ασθενών-μαρτύρων για την Σκλήρυνση Κατά Πλάκας σε Ελληνοκύπριους στην Κύπρο.

Καλείστε να λάβετε μέρος σε μια ερευνητική μελέτη. Προτού αποφασίσετε πρέπει να καταλάβετε γιατί γίνεται η έρευνα και τι ποια θα είναι η δική σας ανάμειξη. Παρακαλώ αφιερώστε λίγο χρόνο για να διαβάσετε προσεκτικά τις παρακάτω πληροφορίες. Υποβάλετε ερωτήσεις εάν κάτι που διαβάζετε δεν είναι σαφές ή επιθυμείτε περισσότερες πληροφορίες. Πάρτε τον χρόνο σας για να αποφασίσετε αν θα συμμετάσχετε ή όχι.

Η μελέτη αυτή είναι βασισμένη σε ερωτηματολόγιο.

- Ποιος είναι ο σκοπός της μελέτης;

Η μελέτη στοχεύει στο να διαπιστώσει ποιοι περιβαλλοντικοί παράγοντες αποτελούν κίνδυνο εμφάνισης Σκλήρυνσης Κατά Πλάκας στους Κύπριους.

Γιατί έχω καλεστεί να συμμετάσχω;
Έχετε επιλεγεί επειδή είστε Ελληνοκύπριος/α. Αυτή η μελέτη δεν απευθύνεται σε άτομα άλλων εθνικοτήτων.
Μερικά άτομα έχουν επιλεγεί επειδή έχουν Σκλήρυνση Κατά Πλάκας. Αυτοί αποτελούν την ομάδα ασθενών.
Άλλα άτομα έχουν επιλεγεί επειδή δεν έχουν Σκλήρυνση Κατά Πλάκας. Αυτοί αποτελούν την ομάδα «μαρτύρων».
Οι περιβαλλοντικοί παράγοντες στους οποίους έχουν εκτεθεί τα άτομα της ομάδας ελέγχου, θα συγκριθούν με εκείνους των ατόμων με Σκλήρυνση Κατά Πλάκας .

Πρέπει να λάβω μέρος;
Όχι. Η συμμετοχή σας στην έρευνα είναι εντελώς εθελοντική. Εάν έχετε Σκλήρυνση Κατά Πλάκας, η παρούσα και η μελλοντική σας θεραπεία θα είναι αναλλοίωτη, ανεξάρτητα από το αν συμμετέχετε ή όχι στην μελέτη. Εάν δεν έχετε Σκλήρυνση Κατά Πλάκας, δεν θα επηρεαστεί οποιαδήποτε άλλη ιατρική θεραπεία που λαμβάνεται τώρα, ή που μπορεί να χρειαστεί να λάβετε στο μέλλον.

Τι θα μου συμβεί εάν λάβω μέρος;
Θα σας ζητηθεί να συμπληρώσετε ένα ερωτηματολόγιο που θα ρωτά για τις διατροφικές συνήθειες και τον τρόπο ζωής σας.
Θα σας πάρει 10-15 λεπτά. Θα σας ζητηθεί να συμπληρώσετε τη φόρμα αυτή μόνο μία φορά και δεν θα επικοινωνήσουμε μαζί σας για οποιαδήποτε άλλη πληροφορία.

Έξοδα και πληρωμή; Δεν υπάρχει πληρωμή για τη μελέτη αυτή.

Τι θα πρέπει να κάνω; Να συμπληρώσετε ένα ερωτηματολόγιο.

Ποια είναι τα πιθανά μειονεκτήματα και οι κίνδυνοι συμμετοχής; Δεν υπάρχουν.

Ποια είναι τα πιθανά οφέλη συμμετοχής;

Δεν υπάρχουν άμεσα οφέλη για εσάς. Παρ ’όλα αυτά, η συμμετοχή σας θα βοηθήσει στον εμπλουτισμό της υφιστάμενης γνώσης για την επιδημιολογία, την παθοφυσιολογία και την επιβάρυνση της σκλήρυνσης κατά πλάκας στον κυπριακό πληθυσμό. Αυτό είναι κάτι που θα είναι εξαιρετικά χρήσιμο για την επινόηση στοχευμένων προγραμμάτων πρόληψης, τα οποία θα είναι πιο αποτελεσματικά και οικονομικά αποδοτικά, μειώνοντας έτσι την επιβάρυνση της νόσου στην κοινωνία και ταυτόχρονα εξοικονομώντας χρήματα από το Εθνικό Σύστημα Υγείας της Κύπρου.

Τι γίνεται αν υπάρξει κάποιο πρόβλημα; Αυτό είναι απίθανο, αλλά εάν έχετε κάποια ανησυχία για οποιαδήποτε πτυχή αυτής της μελέτης, θα πρέπει να ζητήσετε να μιλήσετε με τον ερευνητή Δρ. Paul Johnson ο οποίος θα κάνει ότι μπορεί για να απαντήσει στις ερωτήσεις σας, ή να στείλετε ηλεκτρονικό μήνυμα στη διεύθυνση: Johnson.p@unic.ac.cy

Αν μείνετε δυσαρεστημένοι και επιθυμείτε να διαμαρτυρηθείτε επίσημα μπορείτε απευθυνθείτε στον Δρ. Αλέξανδρο Ηρακλείδη μέσω ηλεκτρονικού ταχυδρομείου στη διεύθυνση: heraclides.a@unic.ac.cy

Θα είναι εμπιστευτική η συμμετοχή μου στη μελέτη;
Όλα τα ερωτηματολόγια θα μετατραπούν σε ανώνυμα και οι συμμετέχοντες θα αναγνωρίζονται από έναν συγκεκριμένο αριθμό μελέτης. Μόνο ο κύριος ερευνητής θα έχει πρόσβαση στο όνομα του ασθενούς και οι πληροφορίες αυτές θα φυλάσσονται, προστατευμένες με κωδικό πρόσβασης, σε ξεχωριστό αρχείο, μόνο στον υπολογιστή του κύριου ερευνητή. Κανένα άλλο άτομο δεν θα μπορεί να συνδέσει ονόματα συμμετεχόντων με οποιαδήποτε πληροφορία που θα συλλεχθεί από το ερωτηματολόγιο.

Όλες οι ηλεκτρονικές πληροφορίες (η βάση δεδομένων) του ερευνητικού έργου θα αποθηκευτούν με ασφάλεια σε ηλεκτρονικό αρχείο με κωδικό πρόσβασης για προστασία. Μόνο ο κύριος ερευνητής θα γνωρίζει τον κωδικό πρόσβασης, ο οποίος κωδικός θα αλλάζεται κάθε 3 μήνες.

Τα συμπληρωμένα ερωτηματολόγια θα κλειδώνονται σε μια ασφαλής θυρίδα φύλαξης στο γραφείο του συντονιστή της μελέτης, στο Ινστιτούτο Νευρολογίας και Γενετικής Κύπρου, αμέσως μετά τη συλλογή των δεδομένων. Μόλις ολοκληρωθεί η μεταφορά από τα ερωτηματολόγια χαρτιού στην ηλεκτρονική βάση δεδομένων, όλα τα ερωτηματολόγια θα αποθηκευτούν με ασφάλεια σε κλειδωμένο μεταλλικό συρτάρι. Κανένα άλλο πρόσωπο εκτός από τον κύριο ερευνητή της μελέτης δεν θα έχει πρόσβαση σε αυτά τα ερωτηματολόγια. Όλοι οι ηλεκτρονικοί υπολογιστές που περιέχουν δεδομένα της μελέτης, θα κλειδώνονται με ασφάλεια στις εγκαταστάσεις του Ινστιτούτου Νευρολογίας και Γενετικής Κύπρου όταν δεν βρίσκονται σε λειτουργία.

Όλες οι πληροφορίες που θα συλλέγονται για εσάς κατά τη διάρκεια της έρευνας θα διατηρούνται αυστηρά εμπιστευτικές και κάθε πληροφορία σχετικά με εσάς που εξέρχεται από το νοσοκομείο / χειρουργείο / πανεπιστήμιο θα έχει το όνομα και τη διεύθυνσή σας καταργημένα, έτσι ώστε να μην μπορεί κανείς να σας αναγνωρίσει.

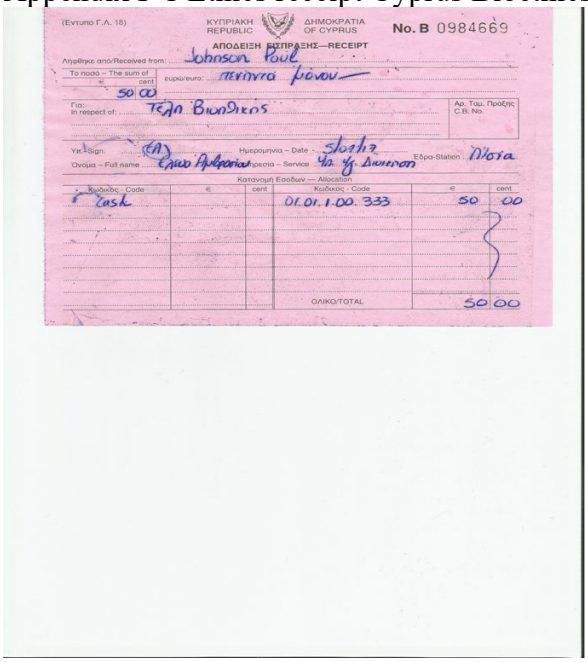
Τι θα συμβεί στα αποτελέσματα της ερευνητικής μελέτης;

Οποιοδήποτε δημοσιευμένο υλικό θα είναι ανώνυμο, ούτως ώστε να μην μπορεί κανείς να σας αναγνωρίσει.

Ποιος διοργανώνει ή χορηγεί την έρευνα;
Η έρευνα αυτή διοργανώνεται από τον Δρ. Paul Johnson, κύριο ερευνητή. Δεν υπάρχει χρηματοδότηση ή χορηγία.

Η μελέτη αυτή έχει εγκριθεί από την Επιτροπή Δεοντολογίας Έρευνας της Σχολής Ιατρικών Επιστημών, μέρος της Επιτροπής Δεοντολογίας Έρευνας του Newcastle University. Η επιτροπή αυτή περιλαμβάνει μέλη τα οποία είναι εσωτερικά της Σχολής, καθώς και ένα εξωτερικό μέλος. Η μελέτη αυτή εξετάστηκε από τα μέλη της επιτροπής, τα οποία πρέπει να παρέχουν αμερόληπτες συμβουλές και να αποφεύγουν σημαντικές συγκρούσεις συμφερόντων.

Appendix F 1 Ethics receipt Cyprus Bioethics


(Έντυπο Γ.Λ. 18) ΚΥΠΡΙΑΚΗ ΔΗΜΟΚΡΑΤΙΑ
REPUBLIC OF CYPRUS
No. B 0984669
ΑΠΟΔΕΙΞΗ ΕΙΣΠΡΑΞΗΣ—RECEIPT
Ληφθηκε από/Received from: Johnson Paul
Το ποσό – The sum of: πενήντα μόνον
€ cent: 50 00
Για: / In respect of: ΤΕΛΗ Βιοηθικής
Αρ. Ταμ. Προόδου / C.R. No.
Υπ. / Sign.
Ημερομηνία – Date: 5/07/17
Όνομα – Full name: Ελένη Αριστοκλέους
Υπηρεσία – Service: Υγ. Υγ. Διοίκηση
Εδρα-Station: Nicosia
Κατανομή Εσόδων — Allocation
Κωδικός / Code: Cash
€ cent:
Κωδικός / Code: 01.01.100.333
€ cent: 50 00
ΟΛΙΚΟ/TOTAL: 50 00

Appendix F 2 Full Ethics Approval University of Newcastle

Faculty of Medical Sciences
Newcastle University
The Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH United Kingdom

University

Paul Johnson
INSTITUTE OF HEALTH & SOCIETY **FACULTY OF MEDICAL SCIENCES: ETHICS COMMITTEE**
Dear Paul,

Title: A Case-Control study of Multiple Sclerosis among Greek Cypriots in Cyprus
Application No: 01269/12905/2017
Start date to end date: 01/05/2017 to 03/09/2018

On behalf of the Faculty of Medical Sciences Ethics Committee, I am writing to confirm that the ethical aspects of your proposal have been considered and your study has been given ethical approval.

The approval is limited to this project: **01269/12905/2017**. If you wish for a further approval to extend this project, please submit a re-application to the FMS Ethics Committee and this will be considered.

During the course of your research project you may find it necessary to revise your protocol. Substantial changes in methodology, or changes that impact on the interface between the researcher and the participants must be considered by the FMS Ethics Committee, prior to implementation.*

At the close of your research project, please report any adverse events that have occurred and the actions that were taken to the FMS Ethics Committee.*

Best wishes,

Yours sincerely

Kimberley Sutherland
On behalf of Faculty Ethics Committee

cc.
Professor Daniel Nettle, Chair of FMS Ethics Committee
Mrs Kay Howes, Research Manager

K. Sutherland *Please refer to the latest guidance available on the internal Newcastle web-site.

tel: +44 (0) 191 208 6000
fax: +44 (0) 191 208 6621
www.ncl.ac.uk

Faculty of Medical Sciences

FMS Ethics Committee

Appendix G 1 Study Debrief Information Sheet-English

STUDY DEBRIEF INFORMATION

This study is a Case-Control study of Multiple sclerosis (MS) among Greek Cypriots in Cyprus. The study aims to establish which environmental factors are a risk of acquiring MS among Cypriots.

This is a questionnaire-based study. Multiple sclerosis is a debilitating disease resulting in Central Nervous System demyelination.

The research questions or hypotheses "Are there risk and protective factors associated with the development of multiple sclerosis in the Cypriot population of Cyprus?"

Through statistical analysis of the data recorded, it is hoped that the environmental risk and protective factors for acquiring MS will be determined.

Thank you for your time. Date……

For further information or any queries, please contact Dr Paul Johnson via e-mail to:
Johnson.p@unic.ac.cy

Appendix G 2 Study Debrief Information Sheet-Greek

ΕΝΗΜΕΡΩΤΙΚΗ ΠΛΗΡΟΦΟΡΗΣΗ ΣΧΕΤΙΚΑ ΜΕ ΤΗ ΜΕΛΕΤΗ

Η μελέτη αυτή είναι μια μελέτη ασθενών μαρτύρων για τη Σκλήρυνση Κατά Πλάκας μεταξύ Ελληνοκυπρίων στην Κύπρο.
Η μελέτη αποσκοπεί να προσδιορίσει τους περιβαλλοντικούς παράγοντες που συνδέονται με τον κίνδυνο απόκτησης σκλήρυνσης κατά πλάκας μεταξύ των Κυπρίων.
Η μελέτη αυτή βασίζεται σε ένα ερωτηματολόγιο.
Η Σκλήρυνσης Κατά Πλάκας είναι μια εξουθενωτική ασθένεια που προκαλεί απομυελίνωση στο Κεντρικό Νευρικό Σύστημα.

Το ερώτημα της ερευνητικής μελέτης είναι: "Υπάρχουν κίνδυνοι και προστατευτικοί παράγοντες που σχετίζονται με την ανάπτυξη σκλήρυνσης κατά πλάκας στον Ελληνοκυπριακό πληθυσμό της Κύπρου;

Μέσω στατιστικής ανάλυσης των καταγεγραμμένων δεδομένων, ελπίζεται ότι θα προσδιοριστούν οι κίνδυνοι και προστατευτικοί παράγοντες που συμβάλλουν στην εμφάνιση Σκλήρυνσης Κατά Πλάκας.

Η μελέτη αυτή έχει εγκριθεί από την Επιτροπή Δεοντολογίας Έρευνας της Σχολής Ιατρικών Επιστημών, μέρος της Επιτροπής Δεοντολογίας Έρευνας του Newcastle University. Η επιτροπή αυτή περιλαμβάνει μέλη τα οποία είναι εσωτερικά της Σχολής, καθώς και ένα εξωτερικό μέλος. Η μελέτη αυτή εξετάστηκε από τα μέλη της επιτροπής, τα οποία πρέπει να παρέχουν αμερόληπτες συμβουλές και να αποφεύγουν σημαντικές συγκρούσεις συμφερόντων.

Ευχαριστώ πολύ για το χρόνο σας.

Ημερομηνία ……
Για περισσότερες πληροφορίες ή οποιεσδήποτε απορίες, παρακαλώ όπως επικοινωνήσετε με τον Δρ. Paul Johnson μέσω ηλεκτρονικού ταχυδρομείου στη διεύθυνση: Johnson.p@unic.ac.cy

Appendix H The Questionnaire This section has the questionnaire in English. The actual questionnaire was in English and Greek.

SECTION 1: DEMOGRAPHICS

Male/Female

Q1.1 What is your date of birth?
Q1.2 **What is your ethnicity ? Greek Cypriot Other**
Q1.3 What is the ethnicity of your mother? **Greek Cypriot Other**
Q1.4 What is the ethnicity of your father? **Greek Cypriot Other**
Q1.5 Which town /city were you born in?

Q1.6 Which town /city do you live in now?

Q1.7 **What is your highest level of education?**

Left after high school, Went to Gymnasium, Went to University but not yet finished, Went to University and graduated

Q1.8 What is your highest qualification ? Please circle only one answer

NONE, GCSE, Apolyterium, BA/BSC

MA/MSC /MBA PHD or similar

Q1.9 What is your current marital status?

A civil partner in a legally-recognised Civil Partnership, Cohabiting, Remarried (second or later marriage), Widowed, Divorced /legally separated, Single/never married, Married (first and only marriage)

Q1.10 What is the ownership status of your current accommodation?

Owned outright by you and/or your partner, Rented from a private landlord, Rented from the council/ local authority, Living rent free with a relative Other

END OF SECTION 1

SECTION 2 SMOKING

Q2.1 Do you currently smoke cigarettes, cigars, shisha water pipe?

Yes /No

If NO. have you you ever smoked cigarettes, cigars, shisha water pipe?
(i) Have you ever smoked as much as one cigarette a day, or one cigar a week, or one ounce (25 grams) of tobacco a month?
Yes No
Instruction ***If NO to BOTH parts of Question 2.1, please go to question 2.12***
Q2.2 Do (did) you inhale the smoke?

Yes/ No

If YES...
(i) Would you say you inhaled the smoke slightly, moderately, or deeply? **(Tick one box)**
Slightly Moderately Deeply
Q2.3 How old were you when you started smoking ? **(Write age in years)**

Years

Q2.4 Do (did) you smoke manufactured cigarettes?
If YES...
(i) How many do (did) you usually smoke per day on **weekdays**?
Per day
(ii) How many do (did) you usually smoke per day on **weekends**?
Per day
(iii) Do (did) you usually smoke plain or filter tip cigarettes?
Plain /Filter

Q2.5 Do (did) you smoke hand-rolled cigarettes?

Yes /No

If YES...
(i) How much tobacco do (did) you usually smoke per **week** in this way?
Per week
(ii) Do (did) you put filters in these cigarettes?
Yes No
Q2.6 Do (did) you smoke a shisha/water pipe?

Yes /No

If YES...
(i) How much shisha pipe tobacco do (did) you usually smoke per **day**?
Per day
Q2.7 Do (did) you smoke small cigars?

Yes /No

If YES...
(i) How many of these do (did) you usually smoke per **day**?
Per day

Q2.8	Do (did) you smoke other cigars?

Yes/ No

If YES...
(i) How many of these do (did) you usually smoke per **week**?
Per week

Have you been cutting down your smoking over the past year?

Yes /No**OR EX SMOKERS (people who smoked in the past but have given up). 2.10**

When did you give up smoking altogether? **(Write age in years) 1**

Years

1. **(a)** I felt it was bad for my health
2. **(b)** I felt it was bad for the health of my family/friends
3. **(c)** It was costing too much money
4. **(d)** Pressure from my family/friends who I live with
5. **(e)** Advice from a doctor or nurse
6. **(f)** I felt it was anti-social
7. **(g)** I was not allowed to smoke at work

How important were the following reasons for you when you gave up smoking? **(Tick one box for each statement)**

Very Important, Important, Fairly Important, Not Important

The following questions relate to your exposure to other people's tobacco smoke

Q2.12	Are you regularly exposed to tobacco smoke from other people smoking around you?
	Yes /No

Where are you regularly exposed to tobacco smoke? **(Tick all that apply)**

At work/ At home ***T HOME***

How many members of your household smoke (or smoked) at home, **excluding yourself**?

END OF SECTION

SECTION 3: ALCOHOL

*Your **current** alcohol consumption.*

I have never drunk (or I tried drinking but didn't pursue it) If you tick this box please go to section 4

Instruction	**If you have NEVER drunk alcohol (or you tried drinking and didn't pursue it), please tick the following box. Otherwise read the guidance below and answer the questions from Q3.1**

In answering this section, please use the following information for guidance:

Never Once or twice a month More than once or twice a week, but not every day

1. **(a)** Spirits (Whisky, Gin, Rum, Brandy, Vodka, Cognac, Ouzo, Zivania)
2. **(b)** sherry, port or vermouth or Commandaria
3. **(c)** Wine (red,white, rose)

One Standard Drink (One Unit)

Half a pint or small bottle (330ml) of Beer/Cider/Lager Half a can or half 660ml bottle of Beer/Cider/Lager small glass (1 x 125ml) of wine, Martini or Cinzano

Half a large glass (1⁄2 x 250ml) of wine, Martini or Cinzano Half a small glass (1⁄2 x 125ml) of Sherry or Port

1 pub measure (1 x 25ml) of Spirits (gin, whisky, vodka etc.) 1 pub measure (1 x 25ml) of liqueur

Q3.1	How often do you typically have a drink containing alcohol?)

Once or twice a week Every day

Glasses per week

Q3.2	In a typical 7-day week, including the weekend, how many **glasses** of the following do you usually have? **(Write in number using the information above for guidance)**

Q3.3 Maximum number of glasses you would drink per day

1. **(a)** Spirits (Whisky, Gin, Rum, Brandy, Vodka, Cognac, Ouzo, Zivania)
2. **(b)** Wine, sherry, port or vermouth or Commandaria
3. **(c)** Beer, lager, or cider
4. (d) Beer, cider

Glasses

Q3.4 How much alcohol would you say that you now drink, compared to earlier in your adult life? **(Tick one box)**

More than I used to Less than I used to

How much wine do you drink per week?

1. **(a) Red wine**
2. **(b) White wine**
3. **(c) Rose**

How long have you been drinking wine?

About the same
I no longer drink alcohol **5**

36 **END OF SECTION 3EF SECTION**

SECTION 4: DIET

*Your diet during the **LAST YEAR**.*

Q4.1 Did you eat any meat or fish in the last 12 months?

Yes/ No . If no go to Question 4.5

Q4.2 What did you do with the visible fat on your meat? **(Tick one box)**

Ate most of the fat, Ate some of the fat, Ate as little as possible

Q4.3 How often did you typically eat grilled or roast meat? **(Write in average frequency, either per week or per month, in one of the spaces provided)**

Times per week **OR** Times per month

Well done / dark brown Lightly cooked / rare

Q4.4 How well cooked did you usually have grilled or roast meat? **(Tick one box)**

Medium
Did not eat meat **Q4.5**

Consumption of **meat and fish** during the **last year**.

Meat and Fish

(Medium Serving)

1. **(a)** Beef: roast, steak, mince, stew or casserole
2. **(b)** Beef burgers
3. **(c)** Pork: roast, chops, stew or slices
4. **(d)** Lamb: roast, chops or stew
5. **(e)** Chicken or other poultry e.g. turkey
6. **(f)** Bacon
7. **(g)** Ham
8. **(h)** Corned beef, Spam, luncheon meats
9. **(i)** Sausages
10. **(j)** Savoury pies, e.g. meat pie, pork pie, pasties, steak & kidney pie, sausage rolls
11. **(k)** Liver, liver pate, liver sausage
12. **(l)** Fried fish in batter, as in fish and chips
13. **(m)** Fish fingers, fish cakes
14. **(n)** Other white fish, fresh or frozen, *e.g. cod, haddock, plaice, sole, halibut*
15. **(o)** Oily fish, fresh or canned, *e.g. mackerel, kippers, tuna, salmon, sardines, herring*
16. **(p)** Shellfish, *e.g. crab, prawns, mussels*
17. **(q)** Fish roe, taramasalata

Q Average use last year **4.6**

Never or < 1 a month, 1 - 3 x a month, 1 per week, 2 - 4 x per week, 5 - 6 x a week, daily, 2 - 3 x a day, 4 - 5 x a day, 6 + a day

Consumption of **bread and savoury biscuits** during the **last year.**
Average use last year

Bread and Savoury Biscuits

(One slice or one biscuit)

1. **(a)** White bread and rolls
2. **(b)** Whole meal bread and rolls
3. **(c)** Cream crackers, cheese biscuits **(**
4. **d)** Crispbread, *e.g. Ryvita*

0 or < 1 - 3 a month, 1 a month, 1 a week, 2 -4 a week, 5 - 6 x a week, daily, 2 -3 x a day, 4 - 5 x a day, 6 + a day

Q4.7 Consumption of breakfast **cereals** during the **last year**.

Breakfast Cereals

(One bowl)

1. **(a) Oat-based cereals such as** porridge, mueseli and Readybrek
2. **(b)** Refined breakfast cereal such as Cornflakes, Rice Crispies, Frosties
3. **(c)** Whole-grained breakfast cereal such as Shredded Wheat, Cherios,Weetabix,Wheat Flakes

Average use last year

0 < 1 a month, 1 - 3 x a month, 1 a week, 2 - 4 x a week, 5 - 6 x a week, daily, 2 - 3 x a day, 4 - 5 x a day, 6 + a day

Write below the top three breakfast cereals you normally consume.

Q4.8 Please estimate your average consumption of **potatoes, rice and pasta** during the **last year**, taking care to complete every line. **(Complete every line - tick one box per line)**

Potatoes, Rice and Pasta

(Medium Serving)

1. **(a)** Boiled, mashed, instant, or jacket potatoes
2. **(b)** Chips
3. **(c)** Roast potatoes
4. **(d)** Potato salad
5. **(e)** White rice
6. **(f)** Brown rice
7. **(g)** White or green pasta, *e.g. spaghetti, macaroni, noodles*
8. **(h)** Wholemeal pasta
9. **(i)** Lasagne, moussaka
10. **(j)** Pizza

Average use last year

0 < 1 a month, 1 - 3 x a month, 1 a week, 2 - 4 x a week, 5 - 6 x a week, daily, 2 - 3 x a day, 4 - 5 x a day, 6 + a day

Average consumption of **dairy products and fats** during the **last year**.
Average use last year

Diary Products and Fats

(Serving Size in Brackets)

1. **(a)** Single or sour cream **(tablespoon)**

2. **(b)** Double or clotted cream **(tablespoon)**
3. **(c)** Low fat yoghurt, fromage frais **(125g carton)**
4. **(d)** Full fat or Greek yoghurt **(125g carton)**
5. **(e)** Daily Desserts, *e.g. chocolate mousse* **(125g carton)**
6. **(f)** Cheese, *e.g. Halloumi, Cheddar, Brie, Edam* **(medium serving)**
7. **(g)** Eggs as boiled, fried, scrambled, etc. **(one)**
8. **(h)** Quiche **(medium serving)**
9. **(i)** Low calorie, low fat salad cream **(tablespoon)**
10. **(j)** French dressing **(tablespoon)**
11. **(k)** Other salad dressing **(tablespoon)**

The following on bread or vegetables

(Teaspoons)

1. **(a)** Butter
2. **(b)** Block margarine, *e.g. Stork*
3. **(c)** Polyunsaturated or olive oil margarine (tub), *e.g. Flora, Vitalite, Bertolli*
4. **(d)** Other soft margarine / dairy spread (tub), *e.g. Clover, Utterly Butterly*
5. **(e)** Low fat spread (tub), *e.g. Flora light, Flora Proactive, Bertolli light*
6. **(f)** Very low fat spread (tub), *e.g. Flora proactiv extra light*

0 or < 1 a month, 1-3 x a month, 2 - 4 x a week, 5 - 6 x a week, 1 a week, daily, 2 - 3 x a day, 4 -5 x a day, 6 +a day

Q4.10	Average consumption of the following **fats (spread onto bread or vegetables)** during the **last year**.

Average use last year

0 or < 1 a month, 1-3 x a month, 2 - 4 x a week, 5 - 6 x a week, 1 a week, daily, 2 - 3 x a day, 4 -5 x a day, 6 +a day

Q4.11	Consumption of **fruit** during the **last year**.

Fruits

(1 Fruit or Medium Serving)

For seasonal fruits such as strawberries, please estimate your average consumption when the fruit is in season

1. **(a)** Apples
2. **(b)** Pears
3. **(c)** Oranges, satsumas, mandarins
4. **(d)** Grapefruit, pomelo
5. **(e)** Bananas
6. **(f)** Grapes
7. **(g)** Melon, water melon
8. **(h)** Peaches, plums, apricots
9. **(i)** Strawberries, raspberries, kiwi fruit
10. **(j)** Tinned fruit

11. **(k)** Dried fruit, *e.g. raisins, prunes, dates, figs*

Average use last year

0 or < 1 a month, 1-3 x a month, 2 - 4 x a week, 5 - 6 x a week, 1 a week, daily, 2 - 3 x a day, 4 -5 x a day, 6 +a day

Q4.12 Consumption of **vegetables (fresh, frozen, or tinned)** during the **last year.**

Vegetables—Fresh, frozen or tinned

(Medium Serving)

1. **(a)** Carrots
2. **(b)** Spinach
3. **(c)** Broccoli, spring greens, kale
4. **(d)** Brussels sprouts
5. **(e)** Cabbage
6. **(f)** Peas
7. **(g)** Capers
8. **(h)** Marrow, courgettes, cucumber
9. **(i)** Cauliflower

Average use last year

0 or < 1 a month, 1-3 x a month, 2 - 4 x a week, 5 - 6 x a week, 1 a week, daily, 2 - 3 x a day, 4 -5 x a day, 6 +a day

Vegetables—Fresh, frozen or tinned

(Medium Serving)

10. **(j)** Parsnips, turnips, Swedes
11. **(k)** Leeks
12. **(l)** Onions
13. **(m)** Garlic
14. **(n)** Mushrooms
15. **(o)** Sweet peppers
16. **(p)** Beansprouts
17. **(q)** Green salad, lettuce, cucumber, celery
18. **(r)** Watercress
19. **(s)** Tomatoes
20. **(t)** Sweetcorn
21. **(u)** Beetroot
22. **(v)** Coleslaw
23. **(w)** Avocado
24. **(x)** Baked Beans
25. **(y)** Dried/tinned lentils, beans, green beans, broad beans, runner beans, black-eyed peas, Fava beans
26. **(z)** Tofu, soya meat, TVP, Veggie-burger

Average use last year

0 or < 1 a month, 1-3 x a month, 2 - 4 x a week, 5 - 6 x a week, 1 a week, daily, 2 - 3 x a day, 4 -5 x a day, 6 +a day

Q4.13 What kind of fat did you most often use for frying, roasting, grilling etc? **(Tick one box)**

Butter

Margarine / dairy spread

Solid vegetable fat

Vegetable /sunflower oil

Olive oil

None

If Olive OIL...

(i)

(i) What type of Olive oil did you use? *E.g. extra virgin, home pressed, etc*

Q4.14 What kind of fat did you most often use for baking cakes? **(Tick one box)**

Butter
Margarine / dairy spread

Solid vegetable fat Vegetable oil

Lard/dripping None

Q4.15 How often did you typically eat food that was fried at home? **(Tick one box)**

Daily, < 1 a week, Rarely, 4-6 x a week, Never, 1-3 x a week

Q4.16 How often did you typically eat food that was fried away from home? *For example, at someone else's home, a takeaway, or at a restaurant?*

Q4.17 How often did you (or whoever usually cooked for you at home) typically add salt to food while cooking?

Q4.18 How often did you typically add salt to any food at the table?

Q4.19 Did you regularly use a salt substitute (*e.g. LoSalt*)?

Brand of salt substitute used?

Q4.20	Have you taken any vitamins, minerals, fish oils, fibre or other food supplements during the past year?

Yes /No /Don't Know

Please list any supplements taken, during the last year *eg Vitamin D, Vitamin C, Cod-liver Oil, Omega 3,*

(a) (b) (c) (d) (e)

Name of Supplement

Dose

(State number of pills, capsules, or teaspoons consumed)

Frequency of consumption (Tick one)

0 or < 1 a month, 1-3 x a month, 2 - 4 x a week, 5 - 6 x a week, 1 a week, daily, 2 - 3 x a day, 4 -5 x a day, 6 +a day

Q4.22	Please list below any foods you avoid and state why.
Q4.23	Please list below any foods you eat more of to help a medical condition and state why?
Q4.24	Have you ever had any specific dietary advice? If yes state from whom?

Yes /No

Q4.25	Please list below any herbs or herbal remedies taken during the last year *eg* St. John's wort (Hypericum perforatum) Ginkgo (Ginkgo biloba). Saw palmetto (Serenoa repens) Ginseng etc

(a) (b) (c) (d) (e)

SOUVLAKI AND SOUVLA

Q4.26-SOUVLAKI AND SOUVLA during the last year.

Souvlaki

Frequency of consumption (Tick one)

or < 1 a month, 1-3 x a month, 2 - 4 x a week, 5 - 6 x a week, 1 a week, daily, 2 - 3 x a day, 4 -5 x a day, 6 +a day

Amount (weight in grams)

Souvla

Other Charcoal grilled meat

Charcoal grilled vegetables

Charcoal grilled fish

How often do you consume organic foods? Never/ Rarely /Often /All the time

End of Section 4

SECTION 5: GENERAL HEALTH WEIGHT HEIGHT

Q5.1 What is your current height without shoes .

Metres

Q5.2 What is your current weight without shoes or clothes.

Kilograms

Q5.3 In the last year have you tried to lose weight but were unable to do so? Yes/No

End of Section 5