The Nutritional Epidemiology of Osteoarthritis and Musculoskeletal Pain.

An analysis of data from two UK population cohorts.

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Abstract

Osteoarthritis (OA) is a leading cause of pain and disability in the UK for which there is, at present, no effective pharmacological treatment and no cure. Given the prevalence of the disease, there is increasing interest in the impact of nutrition and its potential to contribute both to prevention and treatment. Existing data in this area is both inconsistent and limited. This research investigates associations in two large population based cohorts, in cross-sectional and longitudinal settings, with a particular focus on biological pathways known to contribute to disease development.

Data available contained radiographic OA and pain variables in the TWINS-UK cohort, and clinician classified OA and pain variables in the EPIC-Norfolk cohort. Both groups had data on serum lipids and information on dietary exposure obtained through the use of food frequency questionnaires. Genetic data were also available in the EPIC-Norfolk cohort. A cross-sectional analysis was performed in 20517 adults from the EPIC-Norfolk cohort and in 8351 females from the TWINS-UK cohort. Longitudinal analysis investigated incidence and change in hip and knee pain over time in EPIC-Norfolk. Key associations were explored further in a Mendelian randomisation analysis in an attempt to clarify the potential causal relationships between putative exposures and outcomes.

The main finding from the FFQ cross-sectional analysis in TWINS-UK is that a 'Traditional English' dietary pattern showed a 28% increase in the risk of hand OA. An unexpected result was that the 'Fruit and Vegetable' dietary pattern was suggested to increase the risk of knee OA by 19%. Further analysis into the Mediterranean dietary pattern revealed a protective association with OA decreasing the risk of radiographic hand OA by 12%. Analysis of serum lipid levels showed that triglycerides increased the risk of hip OA, hand OA, elbow and forearm pain, and chronic widespread pain. Cholesterol and LDL were also consistently found to increase the risk of OA/pain and the LDL component apoB1 was found to significantly increase the risk of hand OA.

Findings from the case-control and longitudinal analysis in EPIC-Norfolk, revealed that increased BMI is strongly associated with hip and knee OA ($p<0.0001$) and with onset of hip pain ($p<0.0001$) and knee pain ($p=0.0001$). Triglycerides were also found to increase knee OA ($p=0.023$) and knee pain ($p<0.0001$) in the case-control analysis and were found to increase knee pain in females ($p=0.0013$). Findings also showed that cholesterol levels increased the risk of future knee pain ($p=0.016$) and increased vegetable consumption was inversely associated with change in hip pain. The findings from the Mendelian Randomisation study, using SNPs from *FTO* and *APOA5* to investigate reverse causality between BMI and triglycerides and OA, showed no causal associations with OA.

In conclusion, data from these two cohorts suggests that a diet high in saturated fats increases the risk of OA/pain as do the serum levels of lipids. Increased consumption of a Mediterranean diet is suggested to be protective of hand OA. Although BMI is strongly associated with hip and knee OA and incidence and change in hip/knee pain over time, both BMI and triglycerides were found not to be causal of OA when explained by genetic variants responsible for changes in these two exposures. Although wider replication is needed to confirm these observations, the results from this research suggest potential dietary modifications for prevention and treatment of OA and pain associated with OA that could be tested further in randomised trials.

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Publications and Presentations

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Hirst-Jones, K., Hagberg, E., Skinner, J., Guile, G., Cassidy, A., Clark, I., MacGregor, A., 2013. Can diet predict the onset of pain in Osteoarthritis? Poster Presentation, BBSRC Diet and Health Research Industry Club 9th Dissemination Event.

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Hirst-Jones, K., Dainty, J., MacGregor, A., Jones, A., Fraser, W., (In preparation). The effect of a Mediterranean Style Diet on Radiographic Hand OA.

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1 Introduction.

1.1 Chapter Overview

This introductory chapter discusses Osteoarthritis (OA) and the mechanisms that affect its onset and progression. With an increasingly aged and obese population, this particular joint disease is becoming even more of a concern to public health. This chapter outlines the definitions of OA and pain, current treatments, the underlying biological mechanisms and the many risk factors. This is followed by a review of existing evidence for the role of nutrition as a potential preventative treatment, the contribution of known dietary patterns towards health and how lipid metabolism and genetics contribute to OA. OA can result in pain at joint sites which can also influence chronic widespread pain and at present there are limited effective treatments and no cure. Diet has, therefore, become the main focus of research as it is easily modified and has the potential to become a preventative treatment. Identifying dietary risk factors could, therefore, be of great importance for public health.

1.2 The Ageing Population and Musculoskeletal Conditions

The intrinsic process of ageing is continuous, irreversible and consistent with adverse changes which occur over time (Smita, Lange et al. 2016). With 'wear and tear' also seen over time the effects of ageing are masked (van Leeuwen, Vera et al. 2010). A major contributing factor to structural and functional changes that is seen with increased age is cellular damage (Smita, Lange et al. 2016) which leads to reduced physiological integrity with increased vulnerability resulting in deterioration (López-Otín, Blasco et al. 2013). Estimations by the World Health Organisation (WHO) suggest that a quarter of adults aged over the age of 65 years are sufferers of musculoskeletal conditions (Loveless and Fry 2016).

Joint disease and the accompanying musculoskeletal pain are, therefore, a global issue both for patients and health care systems (Nájera, González-Chávez et al. 2016) as are the many different musculoskeletal conditions which display increased incidence and prevalence as the population shows increased longevity. These conditions could be slowed, prevented or even reversed if we were able to gain a better understanding of the underlying mechanisms (Goljanek-Whysall, Iwanejko et al. 2016). A study which followed on from the global burden of disease studies (which investigated non-fatal health outcomes from disease and injuries) found that one of the major contributors to the number of years lived with disability (YLDs), on a global scale, was musculoskeletal disorders. Leading causes for YLDs included lower back pain and neck pain (Vos, Flaxman et al. 2013) and back pain has been found to be present in up to 27% of those aged 20-89 years of age (Loveless and Fry 2016).

Under the umbrella of musculoskeletal conditions, there exists many different types of arthritis: Osteoarthritis, Rheumatoid arthritis, Gout and Spondyloarthropathies (Psoriatic arthritis, ankylosing spondylitis etc.) (Oliviero, Spinella et al. 2015). Typically characterised as inflammation of the joints, all types of arthritis can become extremely debilitating for those who are diagnosed (Arden and Cooper 2005). Osteoarthritis, which is the main focus of this research, is associated with increasing age and BMI with onset typically occurring in

those middle aged. It is on the increase, found worldwide affecting approximately 240 million people (around 10% male and 18% female over the age of 60) (Nelson 2018), is the leading cause of disability in the US (Control and Prevention 2013) and is within the top three reasons for visiting health care providers (Loveless and Fry 2016). It also carries comorbidities with obesity and cardiovascular disease (Oliviero, Spinella et al. 2015). Figure 1 overviews the risk factors and pathogenesis of each of the above forms of arthritis which will be discussed more specifically for OA throughout this chapter.

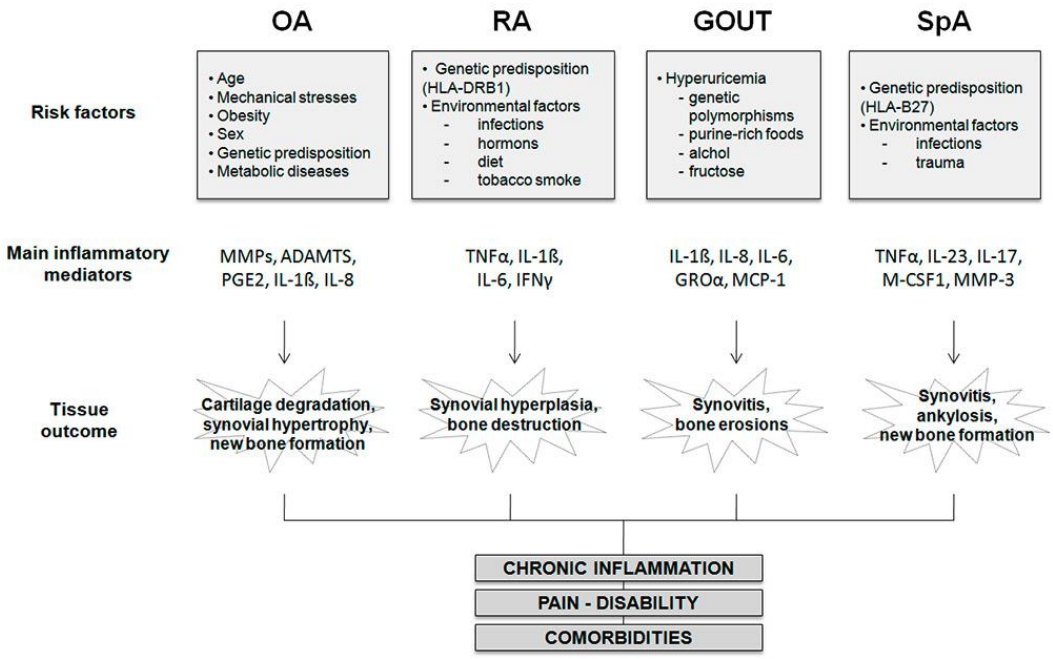

FIGURE 1. OVERVIEW OF THE PATHOGENESIS OF THE MOST COMMON FORMS OF ARTHRITIS (OLIVIERO, SPINELLA ET AL. 2015).

Closely related to many age related diseases is the chronic condition commonly referred to as inflammaging: the characteristic low-grade inflammatory state which develops alongside the ageing process. Inflammaging is now recognised as a key risk factor for disease but despite the abundance of pro-inflammatory markers seen with the inflammaging process, some very elderly, living to over a century, have escaped the onset of disease. Further studies are therefore needed to understand the potential beneficial and detrimental effects of inflammaging and the complex network of mediators and regulators that create an inflammatory response (Cevenini, Monti et al. 2013).

1.3 Defining and Classifying Osteoarthritis

Osteoarthritis can be defined either pathologically, radiographically or clinically for the purpose of investigating the joint disease through epidemiological studies (Zhang and Jordan 2010). The Osteoarthritis Research Society International (OARSI) define OA as "a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic

and/or physiologic derangements (seen in Figure 2 below) which is characterized by cartilage degradation, bone remodelling, osteophyte formation, joint inflammation and loss of normal joint function) that can culminate in illness" (http://oarsi.org/research/standardization-osteoarthritis-definitions).

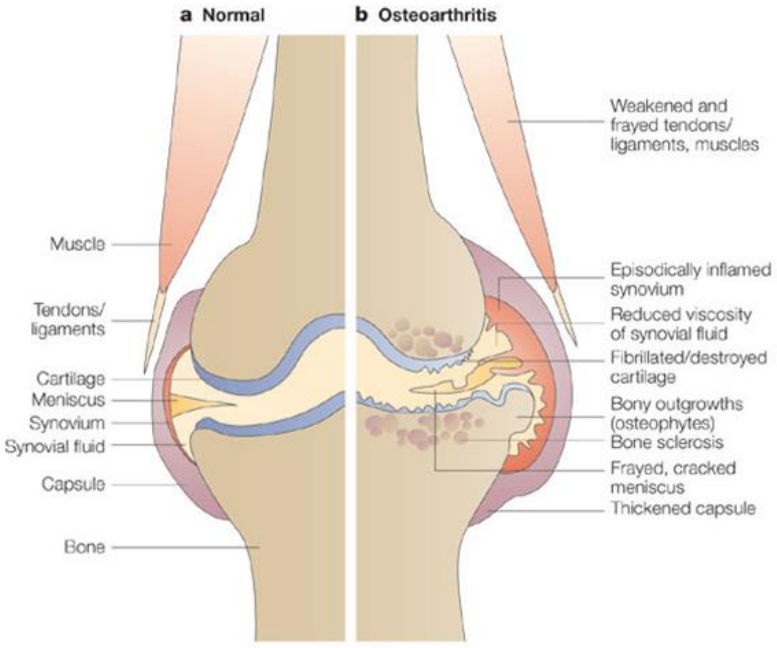

FIGURE 2. A COMPARISON BETWEEN A HEALTHY JOINT AND UNHEALTHY JOINT ENVIRONMENT IN OSTEOARTHRITIS (WIELAND, MICHAELIS ET AL. 2005).

Although multiple ways to define radiographic osteoarthritis exist, the most commonly used method is the Kellgren and Lawrence grading system (Spector, Hart et al. 1993). This scoring system judges the severity of OA and gives a grade based on the advancement of osteophyte formation, joint space narrowing, sclerosis and the destruction of cartilage (Kellgren and Lawrence 1957), discussed further in Chapter 5.3.2.

Due to the aetiology of OA being unclear, there have been different ways in which OA has been defined (Tanna 2004). Clinical definitions suggest that those who suffer joint symptoms and pain may be more relevant as those with radiographic OA may not have clinical joint disease and those that suffer joint symptoms may not have radiographic OA (Zhang and Jordan 2010). Chronic musculoskeletal pain is therefore an important clinical feature that defines OA and there are multiple interactive pathways shown in the biopsychosocial framework (Figure 3) that are believed to determine pain in OA. It is important to consider all biological factors, psychosocial factors (including self-efficacy and pain catastrophizing) and social factors (including social support and pain communication) to be able to improve knowledge and understanding of the pain experience (Hunter, Guermazi et al. 2013).

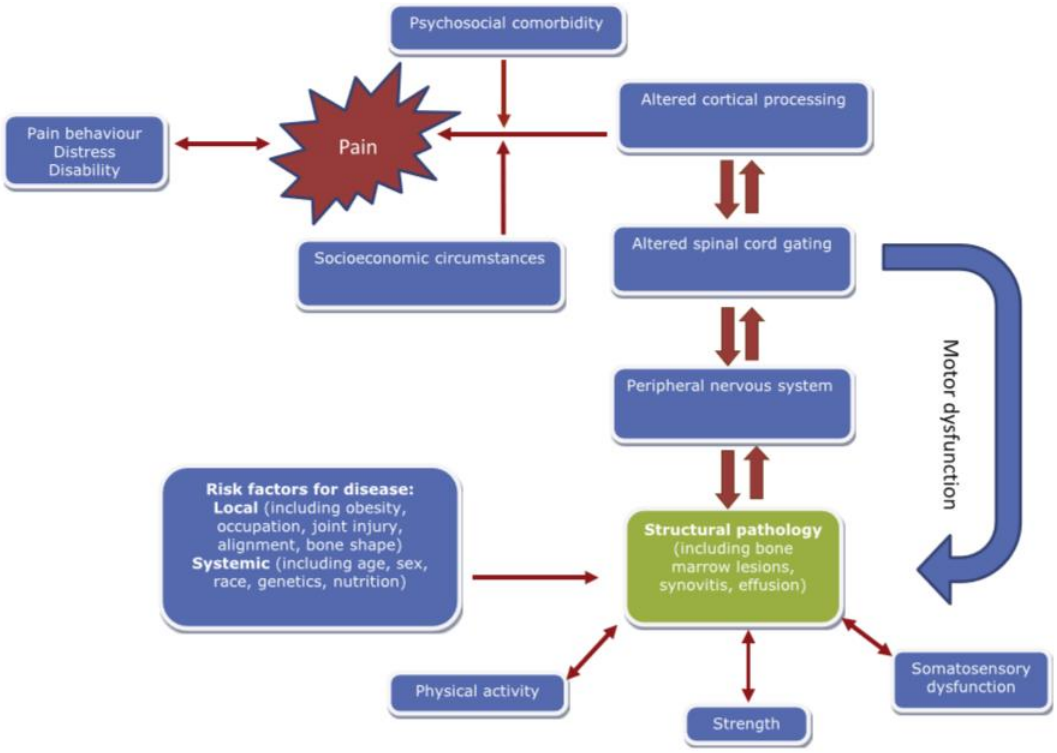

FIGURE 3. MODEL OF THE BIOPSYCHOSOCIAL FACTORS LEADING TO THE EXPERIENCE OF PAIN (HUNTER, GUERMAZI ET AL. 2013).

Chronic musculoskeletal pain affects a third of the adult population and is considered a major public health problem. In order for early intervention and prevention of musculoskeletal pain, the key is to identify the main risk factors involved (Bergman 2007). CWP is defined as “pain of a duration of at least 3 months in an axial distribution, plus pain on both the left and the right side of the body and above and below the waist” (Üçeyler, Valenza et al. 2006). Chronic pain is a clinical condition associated with specific structural disorders (Smith, Macfarlane et al. 2007) such as OA. Studies have investigated CWP by using the stricter ‘Manchester definition’ of CWP (CWP-M) from which findings indicate an impaired health status in those with chronic pain (Bergman 2005).

Osteoarthritis incidence and prevalence has been difficult to assess due to the differences in definitions of the disease. A study that used differing definitions to estimate prevalence in different countries around the world, found that the radiographic definition gave the highest prevalence estimates and that self-reported and symptomatic OA definitions showed similar estimates. This study found that hand OA had the highest prevalence estimates but that knee OA was the most studied (Pereira, Peleteiro et al. 2011) which is consistent with the statistic that 83% of the burden of OA, on a global scale, is due to knee OA (Vos, Flaxman et al. 2013).

A recent global burden of disease study found that the prevalence of knee OA globally is 3.8% with hip OA at 0.85%. There were no substantial changes in these figures between 1990 and 2010 (Cross, Smith et al. 2014). Hand OA continues to be the most common. The US National Health and Nutrition Examination Survey showed a prevalence of 8% and the Framingham cohort showed a prevalence of 7% (Bortoluzzi, Furini et al. 2018). An American study found that hand OA prevalence differed based on ethnicity. Race-specific

symptomatic hand OA risk estimates were 41.4% in those of white ethnicity and 29.2% among African Americans. Gender was found to be increase the risk of developing symptomatic hand OA showing that almost for every one in two women were likely to develop hand OA by age 85 compared to one in every four males. This study also found that the lifetime risk of symptomatic hand OA was increased by 11% among obese individuals (47.1%) compared to non-obese individuals (36.1%) (Qin, Barbour et al. 2017).

Osteoarthritis slowly develops across an individual's lifetime and could therefore be prevented early on in the course of disease progression with regard to improving function and reducing pain, both of which enhance quality of life, alongside slowing the progression of disease (Ageberg and Roos 2015). These are primary targets to be focused on when treating OA in the absence of a cure. Not only intended for alleviating the symptoms of the patient, this approach also aims at reducing the number of cases needing joint replacement in the advanced stages (Bennell, Hall et al.). Clinical practice guidelines encourage and highlight the benefits of a patient driven approach. Key here is the idea that the patient focuses on ways in which they can help themselves in the treatment of OA in a non-drug and non-surgical treatment focused manner (Bennell, Hall et al.).

A good example of this is weight loss, education and exercise in the treatment for degenerative knee disease. Exercise has been reported to reduce pain, improve function and delay the need for joint replacement. Strength training is of importance as muscle weakness is very common in individuals with OA. Greater quadriceps strength for instance protects from the development of OA and muscle strength may be linked to slower progression of OA. Outcomes of exercise as a treatment include improved cartilage matrix quality and improved function and stability as well as reductions in pain (Ageberg and Roos 2015). It has been suggested that to optimise outcomes, programs should be designed for the individual. This is consistent with the general idea of personalised health care during the treatment of OA (Bennell, Hall et al.).

With regard to other interventions and treatments, knee braces aim to improve symptoms and maintain joint structure (by reducing the load on the knee) and show potential for slowing the progression of OA. Needle and laser acupuncture has also been a treatment which can improve pain (Bennell, Hall et al.). Relief from pain by musculoskeletal injections, which can be either corticosteroid injections or local anaesthetic, have also been found to significantly reduce pain (Sucuoğlu, Özbayrak et al. 2016).

Joint replacement is used as a treatment for end stage OA (Wang, Simpson et al. 2011) and is aimed at relieving pain. Joint replacement is seen to be on the increase worldwide (Wang, Simpson et al. 2009), as are prescriptions and visits to specialists and GPs (March and Bagga 2004). These increases are suggested to exist, among other reasons, because of the world wide population's increased life expectancy (Zhang and Jordan, 2010). With this increasing demand it is essential that we build knowledge of potential ways to prevent and/or slow the progression of OA through easily modifiable factors and self-care in an attempt to reduce the demands and increase quality of life for those suffering. Therefore the next part of this chapter discusses the pathological progression and some of the underlying mechanisms to give an overview of what is occurring at a cellular level and to show potential targets for treating OA.

With OA being on the rise and becoming such a major health issue and with there being no known cure at present, this chapter continues to explore the inflammatory pathways and

risk factors for OA of which those that are modifiable, specifically lifestyle factors such as diet, are of great interest.

1.4 Osteoarthritis and Pain: Biological Mechanisms

Pathologically, during the progression of OA, there are many changes within the joint. A few include the destruction and subsequent loss or 'wearing away' of cartilage, appearance of bone marrow lesions and remodelling, laxity of ligaments, stretching of the joint capsule and the weakening of periarticular muscle (Felson, McLaughlin et al. 2003). Synovitis, inflammation, mechanical imbalance and malalignment can then often follow (Pelletier, Martel-Pelletier et al. 2001). As the joint disease progresses through its course, all tissues can be affected and the cross talk between these individual tissues is suggested to be a contributing factor to both the progression of OA and the development of pain (Malfait 2016).

In addition to biomechanical mechanisms, inflammation is now considered an important driver of the molecular mechanism underlying OA/pain. This is due to pro-inflammatory cytokines (such as Interleukin-1 (IL-1) and Tumor necrosis factor (TNF-α)) upregulating the production of factors such as nitric oxide and prostaglandin E2 which induce changes in chondrocytes seen in the progression of the disease (Hedbom and Häuselmann 2002).

Cartilage is composed of a matrix of three major components type II collagen, aggrecan and water which provide the functional properties; smooth articular motion and shock absorption. Type II collagen and aggrecan are lost in the progression of OA and the abundance of the protein is seen to decrease due to the synthesis and activity of matrix metalloproteinases (MMPs) stimulated by chondrocytes when in the presence of a cytokine (Tanigawa, Aida et al. 2011). Chondrocyte proliferation occurs at nearby sites in an attempt to repair the damaged cartilage as a result of signs of focal erosion and ulceration which are associated with cell necrosis. OA results from dysregulation of a number of factors (Arden and Cooper 2005). The biomechanical stresses alongside factors such as pro-inflammatory cytokines and chemokines, pro-inflammatory enzymes (such as MMPs) and changes in the extra cellular matrix (ECM) are all factors to which articular chondrocytes are readily exposed and are therefore subjected to a phenotypic shift. Due to chondrocytes providing the structural maintenance of cartilage, as their primary function is to secrete components of the ECM (Settembre, Arteaga-Solis et al. 2008), the changes in chondrocytes therefore result in a disturbance in cartilage homeostasis (Malfait 2016).

There are many genes and proteins at a molecular level that are involved in the regulation of the environment surrounding the joint. Factors such as pro-inflammatory cytokines and MMPs are in turn regulated by the transcription factor NF-κB and it is suggested that any agent able to suppress these factors and the activation of NF-κB could have the potential to be successful as a treatment (Arden and Cooper 2005). The NF-κB pathway is, therefore, considered a master regulator of immunity. This transcription factor interacts with p50 and p52 NF-κB components and with Rel proteins and resides within the cytoplasm in order to bind to IκB proteins. Upon sensing danger, such as oxidative and genotoxic stress, the IκB proteins are phosphorylated, freeing up and allowing the NF-κB complexes to prompt expression of pro-inflammatory genes within the nucleus. As a result, upregulation of NF-κB has been found to be strongly associated with ageing.

Different physiologic functions of the cyclooxygenases (COX-1 and COX-2) have also been identified by previous research. COX-1 is expressed in the majority of tissues and produces prostaglandins which are important for homeostatic functions. COX-2 however seems more specific in that its expression is very restricted and it is seen to be up-regulated during inflammation. Increased levels have also been seen in patients with rheumatoid arthritis.

Animal models have shown increases in COX-2 to run parallel with increases in prostaglandin production. In vitro experiments, in cell types such as chondrocytes, osteoblasts and synoviocytes, have found increased expression and stimulation of pro-inflammatory cytokines e.g. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) (Crofford 1997). The synthesis of the pro-inflammatory cytokine TNF plays a key role in many diseases and disorders involving inflammation (Van Gool, Galli et al. 2009).

The interleukin family of cytokines alongside the ADAMTS family of catabolic enzymes, both being pro-inflammatory, are now used in lab studies to stimulate, in cell lines, what would normally be seen in the progression of OA in the human joint. Previous research has found *ADAMTS* 1, 5 & 9, part of the NF-κB complex *RELA*, the hyaluronic acid synthase *HAS2* and the heparan sulphate sulfotransferase *HS3ST3A1* to be genes related to ECM turnover and were more highly expressed in OA chondrocytes. With the mRNA reflecting the rate of degradation and synthesis, this study also compared mRNA in the transcriptome of healthy and unhealthy chondrocytes and found that mRNA half-life was shortened in OA chondrocytes (Malfait 2016).

The regulatory system involving the zinc importer ZIP8 and the MTF1 transcription factor has also been investigated. MMP 9, 12 & 13 and ADAMTS-5 expression were all found to be upregulated in the presence of a ZIP8-mediated zinc influx. Overexpression of ZIP8 in mice resulted in OA related changes, such as cartilage damage, however *Zip8* knock out mice showed less of these changes (Kim, Jeon et al. 2014).

The *Silent Information Regulator 2* (*SIR2*) gene was originally found to control genome instability and extend the replicative life span of budding yeast by 30% (Kaeberlein, McVey et al. 1999). *SIR*2 does this by having an impact on the processes associated with ageing such as playing a key role in the silencing of yeast telomeres (Smith and Boeke 1997), preventing recombinant DNA (rDNA) recombination and restricting toxic extrachromosomal rDNA circle formation (Kaeberlein, McVey et al. 1999).

Since the 90's *SIR2*-like genes known as sirtuins, of which there are seven (Frye 2000), have been found to induce changes at a molecular level, that promote health and survival, upon detection of stress, energy intake and daylight. Nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase reactions are carried out by sirtuins (Imai, Armstrong et al. 2000) and, as in the case of SIRT4, also have NAD+-dependent mono-ADP-ribosyltransferase activity (Haigis, Mostoslavsky et al. 2006). Sirtuins are thus categorized by this NAD+ binding and catalytic domain or sirtuin core domain (Frye 2000). Due to the differences in binding to substrates, enzyme activity and patterns of expression sirtuins have very diverse roles (Haigis and Guarente 2006). The sirtuin proteins are distinctive in their activity as they act based on the metabolic state of the cell and the mechanisms by which they act as substrate specific protein deacetylases in order to carry out deacetylation are also unique (Haigis and Sinclair 2010).

SIRT1 has more recently been found to regulate the immune system (Haigis and Sinclair 2010), by way of being able to suppress inflammation (Yoshizaki, Milne et al. 2009) and inhibit transcription of NF-κB (Yeung, Hoberg et al. 2004). It has been found to bind to the aforementioned Rel proteins, specifically RelA/p65, which halts NF-κB transcription (Yeung, Hoberg et al. 2004). Sirtuins may also indirectly regulate NF-κB by interacting with FOXO proteins which can inhibit TNFα-induced activation of NF-κB (Peng 2007). Importantly, for this study, SIRT1 has been shown to impact on obesity and other metabolic disease due to it affecting lipid accumulation in adipocytes and supporting this was the finding that increased inflammation and expression of NF-κB existed in liver specific-SIRT1 null mice. This was due to the activity of peroxisome proliferators-activated receptor alpha (PPARα) which is involved in the response to fasting and in the absence of SIRT1 which is known to suppress PPARα signalling (Purushotham, Schug et al. 2009).

Studies have, therefore, suggested that SIRT1 has beneficial effects on metabolic disorders and the associated state of chronic inflammation due to its ability to stop inflammation in adipocytes and macrophages. Lipid-induced inflammation was found in lower levels in a study that investigated *SIRT1*-overexpressing (and resveratrol treated) mice which showed reduced NF-κB activity and decreased TNFα and IL-6 expression (Baur, Pearson et al. 2006). These two pro-inflammatory cytokines, TNFα and IL-6, were found to be associated with change in pain whilst in a standing position and changes in TNFα were positively associated with change in total knee pain.

Another inflammatory marker positively associated with a change in knee pain whilst in a sitting position or lying down was high sensitivity CRP (Stannus, Jones et al. 2013). SIRT6 is another sirtuin linked to ageing, genome stability and the immune response (Mostoslavsky, Chua et al. 2006). SIRT6, similarly, has been shown to interact with Rel proteins and stop expression of NF-κB targets (Kawahara, Michishita et al. 2009). SIRT6 null mice have showed increased signs of ageing (Mostoslavsky, Chua et al. 2006) along with other defects and increased expression of NF-κB targets (Kawahara, Michishita et al. 2009). SIRT6 has also been shown to regulate TNF production (Van Gool, Galli et al. 2009).

As a result of the suggestion that inflammation plays a key role in OA, studies have focused their efforts on biomarkers of tissue inflammation in relation to the OA. The Rotterdam study investigated factors such as CRP found to be released from tissues by the activity of MMPs. This study found that biomarkers of tissue inflammation such as MMP-dependent degradation of CRP, the collagen biomarker C1M (a measure of soft tissue degradation) and established biomarkers of OA progression such as serum cartilage oligomeric protein (COMP) were found to be positively associated with progression of OA confirming that inflammation has pathological relevance in the disease (Hosnijeh, Siebuhr et al. 2016).

Those biological mechanisms of importance when looking at the development of chronic pain include neurological, endocrine and immunological and genetic mechanisms (Smith, Macfarlane et al. 2007). Pain is produced by nociception where stimulation is repeated on unmyelinated and small myelinated fibres which processes stimuli that damage the joint and surrounding tissue (Felson 2005). Mechanoreceptors and nerve endings in the surrounding structures of the joint are then stimulated, excluding cartilage, resulting in the origin of joint pain (Haviv, Bronak et al. 2013). There is an absence of nociceptors and pain fibres in cartilage (Felson, Chaisson et al. 2001) which supports and gives an explanation for those suffering from radiographic OA, who have been diagnosed and given a Kellgren-Lawrence score, but may not be able to feel pain (Zhang and Jordan 2010). As one the main

structural changes in OA is cartilage degradation, other potential explanations are discussed below as to why pain is felt and can become so severe

Many other tissues affected by OA do have pain fibres present (Felson, Chaisson et al. 2001) and inflammation lowers the threshold for nociception. Nociceptive fibres are found within the bone marrow and bone in the periosteum and bone marrow lesions (BMLs) have been shown to be strongly associated with the presence of pain in knee osteoarthritis (Felson, Chaisson et al. 2001).

Osteoarthritic pain is diagnosed as either acute or chronic, however, the progression from acute to chronic pain is not well understood. Acute pain, which is a result of stimulation of mechanoreceptors and pain receptors, is believed to be protective by acting as a warning of continuous tissue damage and is a symptom of the disease process. Chronic pain, however, is a disease process where activation of N-methyl-D-aspartate (NMDA) receptors stimulates release of the neuropeptide substance P which has been found within many tissues in the joint and is thought to be involved in the activation of inflammatory cells and secretion of inflammatory mediators (Allen 2011). Knee pain is suggested to be due to inflammation within the synovium, potentially due to synovial fluid escaping due to swelling of the joint capsule (Haviv, Bronak et al. 2013)), putting pressure on the nerve endings, ligaments and joint capsule (Cuzdan Coskun, Ay et al. 2015). The growth of new nerve fibres have been seen to penetrate cartilage and osteophytes, which is helped by angiogenesis and which are also involved in mediating the pain described by osteoarthritic patients.

Chronic peripheral pain stimuli relating to OA is suggested to lead to hyperexcitability of neurons (Kosek and Ordeberg 2000). Pain experienced that is not necessarily due to osteoarthritic changes can be a result of convergence pain where pain surrounding the knee originates from a different source due to nociceptors converging on the same pain projection neurons. Pain can also be due to tissue injury and problems with the regulation of the transmission of pain. Tissue repair eventually follows nerve sensitization but some individuals experience persistent pain due to transport of substance P within the neurons which is assisted by COX-2 upregulation. Therefore in patients with radiographic OA, the origin of pain can be due to many factors and not solely to radiographic features (Allen 2011).

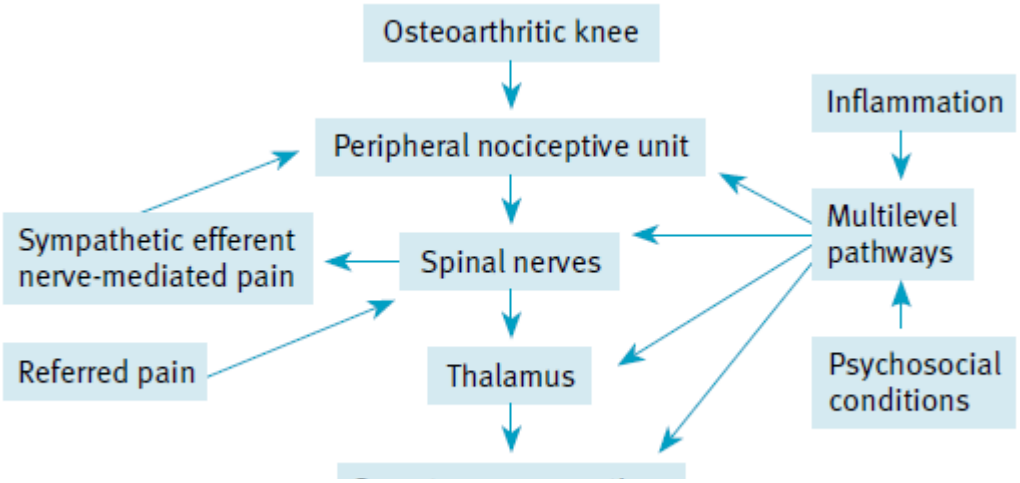

FIGURE 4. PAIN TRANSMISSION PATHWAY SHOWING THE MOVEMENT OF PAIN SIGNALS FROM AN OSTEOARTHRITIC KNEE (HAVIV, BRONAK ET AL. 2013).

Central sensitisation is the process by which central pain processing pathways become more sensitive due to pain-inhibitory mechanisms malfunctioning and altered sensory processing in the brain. As a result of increased reactivity in the central nervous system, the regulatory pathways for the autonomic, endocrine and immune system can become disrupted. The nociceptive system is controlled by pain modulating pathways that suppress surrounding neuronal activity. In the case of central sensitisation and CWP there is a malfunctioning of these pain inhibitory pathways. For peripheral and central sensitisation to take place an ongoing source of pain is needed. Damage or inflammation of peripheral tissues that inflicts pain is capable of causing CWP (Meeus and Nijs 2007).

There are several hypotheses regarding the pathophysiology of CWP including that of the potential role of cytokines. Levels of anti-inflammatory cytokines have been found to be reduced in patients with CWP (Üçeyler, Valenza et al. 2006). Previous research has found that, in the induction of neuropathic pain, there was a role for tumor necrosis factor-alpha (TNF-α) which activates p38 mitogen-activated kinase. (Schäfers, Svensson et al. 2003). Many studies have shown that pro-inflammatory cytokines are involved in the development of both neuropathic and inflammatory pain in both their ability directly (receptor-mediated) and by involving further mediators (Sommer and Kress 2004).

1.5 Risk Factors of Osteoarthritis and Pain

Having discussed OA and pain, as the main focus of this research, as well as the potential biological mechanisms underlying these, this chapter will overview to what extent the pathways and mechanisms at a cellular level are reflected in risk factors of OA/pain.

A review into the epidemiology of OA identified aetiological factors involved in the development of the joint disease as old age, female gender, obesity, bone density, muscle weakness, joint laxity and injury to or repetitive use of joints (Zhang and Jordan 2010). Genetic factors also have an important contribution to the joint disease with regard to cartilage loss (Thakur, Dawes et al. 2013) and, specifically with regard to hand OA, it has been suggested that the effects of genes may be specific to the different pathways of pathological progression (Doherty, Spector et al. 2000).

Although the aetiology is still not fully understood, age does appear to be the biggest contributing factor as the greatest incidence of OA is seen in women aged 50-59 years and in men aged 60-69 years (Sun, Gooch et al. 2007). Population based radiographic surveys also revealed that the prevalence of OA, whilst varying with geographical location, rises with age at all joint sites (Arden and Cooper 2005) and is found worldwide with prevalence of knee osteoarthritis having been reported at 64% for both genders and 74% for females (Vos, Flaxman et al. 2013). However, OA can also occur in those of younger age, specifically knee OA, where traumatic injury (such as ligament rupture or meniscal tears) has occurred in the past, making previous injury a risk factor for accelerated development of knee OA (Ackerman, Kemp et al. 2017). A study looking into American athletes found that partial meniscectomy and reconstructive surgery of the anterior cruciate ligament in particular, are associated with knee OA in the elite (Smith, Nepple et al. 2016). Early onset of hip OA, however, can be seen when the patient has experienced developmental complications such as structural deformities e.g. femoroacetabular impingement syndrome (Ackerman, Kemp et al. 2017). Already having joint disease also seems to be a contributing factor and potentially influences OA at other sites. Hand OA, for instance, has been found to be

related to the occurrence of OA at other sites and was found to be strongly associated with the prevalence of total knee replacement, alongside BMI (Jonsson, Helgadottir et al. 2011).

Obesity, measured by BMI, is also a major risk factor for OA (Elliott, Chapman et al. 2013). Studies have found a statistically significant association between radiographic hand OA and BMI and confirms that seen in many others. The relationship between hip and knee OA and an elevated BMI was traditionally thought to be due to the excess weight having a negative impact on the joint (Holliday, McWilliams et al. 2011). There are many studies and reviews surrounding weight management and the relationship between obesity and OA (De Luis, Izaola et al. 2012).

Ethnic groups are another suggested potential risk factor due to differing results from cross-sectional studies. Hip OA, for instance, has been found to be lowest in Asians and highest in white Europeans (Lievense, Bierma‐Zeinstra et al. 2002). Dietary magnesium intake has been found to be associated with radiographic knee OA but this association was not found in African American ethnic groups (Qin, Shi et al. 2012). These differences could be due to variations in lifestyle factors such as diet. As a part of dietary patterns moderate alcohol consumption has been found to be associated with a higher KL grade and an increase in severity of OA but is was also significantly associated with double the odds of erosive hand OA (Haugen, Magnusson et al. 2017). This, alongside smoking, is a lifestyle choice and is a modifiable risk factor.

Smoking is suggested to be pro-inflammatory but despite the success of campaigns it still remains prevalent (Dean and Gormsen Hansen 2012) and has been found to be linked with inflammation associated with OA (Amin, Niu et al. 2007). Male OA patients who smoke, for instance, experience more knee pain and cartilage loss than non-smoking males (Amin, Niu et al. 2007). Symptoms and function in older adults with knee OA has been seen to improve with moderate levels of physical activity such as yoga and aerobic/strengthening exercises (Cheung, Wyman et al. 2016) suggesting that the modifiable 'inactive lifestyle' risk factor also plays a key role in OA (Dean and Gormsen Hansen 2012).

Many studies have produced differing results between males and females suggesting that gender is also a risk factor for OA. An epidemiological study including both males and females, which assessed the prevalence of mild and severe radiological OA in 22 joints, found that a higher proportion of severe radiological OA in most joints was found in females (Van Saase, Van Romunde et al. 1989). Females also seemed to have greater risk of knee OA in a GOAL study that looked at associations with body shape in each decade of life (Holliday, McWilliams et al. 2011). Hormones may explain these differences as they are thought to play a role in the development of OA due to the observed increase in OA in women at the time of menopause (Zhang and Jordan 2010). Higher rates of radiographic knee OA cases have also been found in women who had previously undergone a hysterectomy compared with those who had not (Spector, Hart et al. 1991). OA symptoms in females are typically seen around menopausal age and is more pronounced in the hand as opposed to the hip and knee joints (discussed further in Chapter 8). Research has found that women who have undergone ovariectomy or pharmacologically induced menopause experience increased knee and hand OA (Watt 2016).

The risk factors affecting the progression of OA such as obesity, joint injury and physical activity all appear to be joint specific. A factor, therefore, might show no association with OA of one joint but could show an association with another (Arden and Cooper 2005).

Looking specifically within knee OA at the early stages of disease, results (using an elderly population from the first cohort within the Rotterdam Study) showed that genetic and biomechanical markers alongside questionnaire variables and the presence of OA at other joint sites did not predict knee OA incidence as strongly as minor radiographic degenerative X-ray features could. This has been suggested to be a hugely important finding due to the very minor and doubtful changes rarely being recorded by radiologists (Kerkhof, Bierma-Zeinstra et al. 2014).

Epidemiological studies over the last decade investigating chronic widespread musculoskeletal pain have found higher rates of complaints of this major health problem among women (Gran 2003). A study published in 2005 revealed different risk factors associated with musculoskeletal pain in men and women over the age of 72. From this Framingham study, pain in one or more regions was reported in 63% of women compared to 52% of men and widespread pain was prevalent in 15% of women compared to 5% of men (Leveille, Zhang et al. 2005).

More recent studies have demonstrated that women are at substantially greater risk for many clinical pain conditions (Fillingim, King et al. 2009). A Swedish study, conducted over a 3 year time period, looking at the development and persistence of chronic widespread pain investigated three groups (those with no chronic pain, chronic regional pain and CWP. Findings were that risk factors varied amongst individuals. A family history of chronic pain and higher age group was shown to predict the development of CWP but having personal social support and a habit of drinking alcohol on a weekly basis was shown to be protective (Bergman, Herrström et al. 2002).

Pain has also been shown to be associated with disability, poorer health, and a history of back pain prior to 65 years of age in both men and women. BMI, systolic blood pressure and symptoms of depression were associated with pain in women but in men pain was associated with radiographic OA (Leveille, Zhang et al. 2005). CWP was shown to be associated with mortality in a study that followed a cohort over 12 years and showed higher rates of mortality in a group initially reporting CWP as opposed to other groups (Andersson 2004).

The results of a study that investigated health status in the general population aged 20-74 years, found that physical and mental health status are affected by musculoskeletal pain and predict the further development of pain (Bergman, Jacobsson et al. 2004). Other studies have investigated the strong association between CWP and psychosocial distress in particular to assess the impact of various factors on the development of new cases of CWP. Findings were that individuals that showed features of somatization, health seeking behaviour and poor sleep were at substantially increased odds of developing CWP (Gupta, Silman et al. 2007). In most cases pain is persistent in patients in clinic but in community derived patients only half experienced persistent pain and it was predominantly those with psychological distress. Studies have indeed showed that individuals are more likely to have persistent widespread pain when showing features of somatization and that prevalence increases with greater exposure to risk factors (McBeth, Macfarlane et al. 2001).

Lower back pain and neck pain have both been reported as a consequence of previous disease/injury (Vos, Flaxman et al. 2013). Studies investigating chronic widespread pain using the stricter definition CWP-M found that factors such as education level, lower socio-economic group, lower social support, immigrant status and living in a compromised

residential area all associated with CWP (Bergman 2005). Loneliness is also an established risk factor for musculoskeletal pain (Docking, Fleming et al. 2011) based on the theory that in socially isolated areas there is greater reporting of symptoms due to the lack of distractions (Docking, Beasley et al. 2015). It is also suggested that personality could be important as a recent study found that high neuroticism and low conscientiousness were associated with a high level of chronic pain (Bucourt, Martaillé et al. 2016). CWP-M was also found to be associated with psychological disturbance, low levels of self-care and the reporting of other symptoms (Hunt, Silman et al. 1999). CWP is also linked to fatigue and these are two of the symptoms involved in the diagnosis of Fibromyalgia which has been found to be associated with workplace stress, bullying in the workplace and high workload suggesting that stress is an important contributing factor towards the development of pain (Kivimaki, Leino-Arjas et al. 2004).

It is therefore important to consider these many factors involved in the development of OA and pain when conducting an investigation into pain associated with OA, as there are a number of factors that could potentially be involved in the development and progression of the disease. Having discussed how lifestyle factors can influence OA and how they act as risk factors for OA/pain, the next chapter will overview nutrition and why it has become such a major focus of OA research.

1.6 The Evidence for the role of Nutrition in the development of Osteoarthritis

Due to there being no known cure for OA, prevention is therefore of great importance and it is suggested that diet could be key to this. Dietary manipulation is one of the ways in which patients feel they can help themselves to relieve symptoms and the most common dietary approaches are vegan, vegetarian, Mediterranean and elemental diets (Rayman and Pattison 2008). The literature surrounding diet suggests that consumption mainly of meat with the use of animal fats combined with the typical lifestyle seen in a western culture (Landaeta-Diaz, Fernandez et al. 2013) is pro-inflammatory (Dean and Gormsen Hansen 2012). In contrast, a diet which is plant based, rich in fibre and fruit and vegetables and which favours olive oil (Landaeta-Diaz, Fernandez et al. 2013), seen typically in Mediterranean and African lifestyles, is suggested to be anti-inflammatory (Dean and Hansen, 2012).

Nutrition has, therefore, become the main focus of research for its potential as a preventative treatment for OA. The theory that nutritional compounds have a 'build up' effect over time and therefore chronic diseases should benefit more from this than acute diseases (Ameye and Chee 2006) has been explored by many. In the past the idea of dietary manipulation has been dismissed by sceptic rheumatologists mainly due to a lack of evidence (Rayman and Pattison 2008). This chapter explores the current literature on diet and nutrition in relation to OA/pain with a focus on the Mediterranean style diet and the plant based compounds found within this dietary pattern.

1.6.1 Mediterranean Diet and Hormesis

The Mediterranean diet (Med diet) is reflective of the dietary pattern found in Mediterranean countries such as Greece, Italy and Spain in the 1960s (Rayman and Pattison 2008). This dietary pattern is now considered an established healthy eating behaviour (Veronese, Stubbs et al. 2016) and is considered to be more of a way of life rather than simply a dietary pattern (Estruch and Bach-Faig 2018). The Med diet is rich in long chain ω-3 PUFAs, nutrients known to have antioxidant properties and unrefined carbohydrates (Rayman and Pattison 2008) as it is made up of foods that are largely plant based (fruits, vegetables, wholegrain cereals, potatoes, beans, nuts and seeds) with locally sourced seasonal foods that have undergone minimal processing (e.g. fish and olive oil) as the main sources of fat. Low to moderate amounts of dairy products (mainly cheese and yoghurt), meat and meat products (poultry, eggs and red meat) and alcohol (usually in the form of red wine) are also found within this dietary pattern (Willett, Sacks et al. 1995). These foods characteristic of the Med diet are referred to as 'functional foods', known to have positive effects on health and wellbeing (Oliviero, Spinella et al. 2015).

Changing an entire dietary pattern (as opposed to incorporating foods into an existing diet) is suggested to be a better approach to take in order to gain the benefits from a Mediterranean style diet. Multiple processes are targeted as a result of consuming a Med diet, so investigating the health impacts from the dietary pattern as a whole as opposed to evaluating the impact of single nutrients on the inflammatory process (Cevenini, Monti et al. 2013) is also suggested to be a better approach as synergistic effects of compounds within the dietary pattern are taken into account. This 'whole diet' approach is a strength of the NU-AGE dietary intervention study which found that adopting a Med diet is beneficial for improving the health of adults (Berendsen, van de Rest et al. 2018).

The most reported and widely discussed beneficial effect of consuming a Mediterranean diet is on inflammation (Oliviero, Spinella et al. 2015). Adhering to a Med diet has been shown to be associated with healthy ageing in those middle aged which suggests that this dietary pattern would be a good option for maintaining good overall health (Assmann, Adjibade et al. 2017). Adherence to the Med diet has been shown to reduce the incidence of cardiovascular disease by approximately 30%, type-2 diabetes by 40% and metabolic syndrome by approximately 10% (Estruch, Ros et al. 2013). Some studies have drawn associations between a Med diet and reduction in cancer risk (Barak and Fridman 2017), however this conflicts with studies such as those using the EPIC cohort (Molina-Montes, Sánchez et al. 2017) so further research is needed to understand the effects of a Med diet on cancer.

It has also been suggested that a diet rich in the foods found within the Med diet is protective of Alzheimer's disease and Dementia (Solfrizzi, Frisardi et al. 2011) and of other neurodegenerative diseases (Liu, Gao et al. 2019). Results from the SUN project showed that adherence to the Med diet is associated with a better self-perceived mental and physical health related quality of life (Sánchez, Ruano et al. 2012). Other studies have shown that high adherence to this diet is related to better academic performance mediated by quality of sleep (Adelantado-Renau, Beltran-Valls et al. 2019) and shows protective effects for mood and mental health (Albaladejo-Blázquez, Ferrer-Cascales et al. 2018) for instance reduced odds of depression, anxiety and psychological distress (Sadeghi, Keshteli et al. 2019). Longitudinal studies have found that the Mediterranean diet is associated with lower incidence of frailty ('increased vulnerability to stressors causing limited capacity to

maintain homeostasis'). This in turn affects the number of hospital admissions and institutionalisations usually seen with the transition from frailty to disability which implies that consuming a Med diet could indirectly reduce these adverse outcomes within a population (Veronese, Stubbs et al. 2018).

When exploring OA specifically studies are limited with most being of cross-sectional design and with a focus on knee OA. Those studies that have investigated OA have found that adherence to a Med diet is associated with a better quality of life in terms of decreased pain, disability and symptoms of depression (Veronese, Stubbs et al. 2016). Cross-sectional studies have found that adherence to the Med diet is associated with lower prevalence of knee OA (Veronese, Stubbs et al. 2017). It is worth noting that in these studies the consistent factor was that those participants that had higher adherence to the Med diet also had lower BMI's, were in good health medically speaking and had a higher level of education. A study that used MRI assessment to investigate knee cartilage found that high adherence to a Med diet is associated with a significant improvement in knee cartilage (Veronese, La Tegola et al. 2018) and a longitudinal study found that a Med diet is associated with a lower risk of radiographic symptomatic knee OA. Those with high adherence also reported lower risk of pain worsening (Veronese, Koyanagi et al. 2018). Although very few studies have investigated the association between adherence to a Med diet and OA a recent systematic review concluded that despite the lack of epidemiological evidence and the need for more long term interventions there does appear to be a relationship between adherence to a Med diet and OA (Morales-Ivorra, Romera-Baures et al. 2018).

Despite the research emerging showing the beneficial effects of the Med diet, adherence to this dietary pattern is threatened by socioeconomic and lifestyle factors (Estruch, Ros et al. 2013). For example items within the Med diet have a relatively low cost and are readily available in southern European countries but are only available to those within higher socioeconomic classes in northern European countries due to the increased cost (Veglia, Baldassarre et al. 2019). However, even in those traditional areas most suited to this dietary style, fewer people are adhering to it (Rizza, De Gara et al. 2016).

Adherence to a Med diet has been investigated due to being particularly poor in young adolescents, who are perhaps influenced by the temptations of the modern westernized world. Fast paced lifestyles and successful marketing strategies result in consumption of highly processed snacks and fast foods. It has also been shown that greater feelings of loneliness result in poor adherence to the Med diet (Ferrer-Cascales, Albaladejo-Blázquez et al. 2018) and an addictive eating pattern high in sugars, salts and fats ensues. This association is thought to be indirect due to loneliness increasing stress levels and therefore the influential relationship between stress and eating behaviours becomes relevant (Ferrer-Cascales, Albaladejo-Blázquez et al. 2018). Despite the Med diet having been recognised as promoting quality of life and lifelong wellbeing and now standing as a recommendation for primary and secondary prevention of major chronic diseases (Lichtenstein, Appel et al. 2006), there still exists an increasing trend in a shift towards vegetarian, vegan and raw dietary options. Despite the available foods in Mediterranean countries the shift away from this traditional dietary pattern suggests that this diet needs adaptation in order to be a feasible option alongside new lifestyle, agricultural and environmental constraints (Estruch and Bach-Faig 2018).

With the Med diet being made up mostly of plant based foods, it is important to understand and consider how, by consuming a Med diet, these foods confer a beneficial

effect. Polyphenols are natural chemicals produced by plants in response, and by way of adapting, to the stresses of the environment. Having the ability to move is a luxury plants do not possess however they are still able to avoid extreme exposure, dehydration and resist attack so the response to stress, although chemically similar to ours, is more complex and superior (Hooper, Hooper et al. 2010). Hormesis is the name given to the process whereby mild amounts of stress are undergone in small doses by being exposed to those factors which would normally be harmful if taken to the extreme (Rattan 2008). In 1538, Paracelsus was the first to theorise about this phenomenon known as mithridatism: "All things are poison and not without poison; only the dose makes the thing not a poison". This was followed by Schulz in 1887 who demonstrated that there can be opposite effects from a toxic substance dependent on the dose. Pre-conditioning is the term given to the process whereby mild stressors activate and upregulate defence pathways at a cellular and molecular level. The successive adaptation and protective properties that this process results in is named post-conditioning. The hormesis paradigm conceptualises caloric restriction which is the most studied area within the area of nutrition and ageing (Martucci, Ostan et al. 2017).

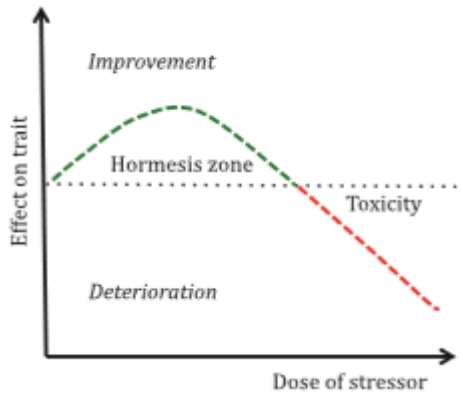

FIGURE 5. DOSE RESPONSE CURVE AS A RESULT OF HORMETIC STIMULI

The stressors stimulate the cellular stress response and other pathways of repair in order to improve health, fitness and adaptability to our environment (Rattan 2008). These mechanisms include inflammation, antioxidant system activation, autophagy, heat shock protein response, unfolded protein response, DNA damage response and sirtuin response (Martucci, Ostan et al. 2017).

There has been a lot of epidemiological research that shows that diets rich in flavonoids (a sub class of polyphenols) are associated with lower incidence of disease (Lu, Xiao et al. 2013). Flavones, Flavonols, Flavanones, Flavanols, Isoflavones and Anthocyanidins are all classifications of Flavonoids in which the chemical structure contains at least one sugar group bound to phenolic groups by glycosidic linkages (see Figure 6 below) (Kinoshita, Lepp et al. 2006). Flavonoids are the most studied class (Chaaban, Ioannou et al. 2017) and there have been more than 6000 different flavonoids found. These are widely distributed in plants with hundreds having been found in edible plants (Falcone Ferreyra, Rius et al. 2012). Flavonoids are believed to play a role in preventing disease due to their strong antioxidant properties as ROS are heavily involved in the pathogenesis of disease. However, it has been suggested that beneficial effects of flavonoids could be indirect. Their abilities are likely to include filtering UV light and acting as signalling molecules due to the low

bioavailability of exogenous flavonoids. Indeed many of the health benefits we see from consuming flavonoids can actually be explained biologically by the functions of their targets (Lu, Xiao et al. 2013).

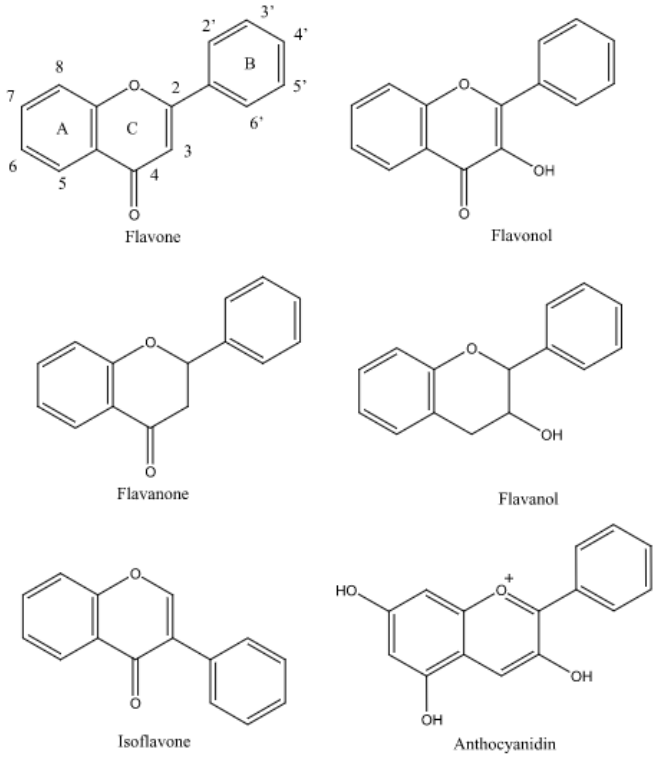

FIGURE 6. THE CHEMICAL STRUCTURES OF FLAVONOIDS (KINOSHITA, LEPP ET AL. 2006).

It is suggested that the best sources of polyphenols are those foods grown in the wild where they have undergone more stress than those grown under controlled and 'ideal' conditions to increase yield (Hooper, Hooper et al. 2010). This is likely due to the effects of light and oxygen on antioxidant activity. It has been found that flavonoids in raw food are stable to light due to their protection by the food matrix whereas in processed foods flavonoids experience photo degradation although research is still to understand more about the effects of light and oxygen on antioxidant activity (Chaaban, Ioannou et al. 2017)

Foods that seemingly produce high levels of polyphenols are highly recommended in diet plans and have gained the name SIRT foods for their ability to activate a family of genes called sirtuins. These are referred to as master regulators (Li 2013), due to sirtuin activity having been found to show a clear association with cell metabolism (Ghosh, George et al. 2010). With the intake of a Med diet there are multiple mechanisms that are likely to be responsible for the resulting protective effect (Veglia, Baldassarre et al. 2019).

The next section of this chapter outlines in depth previous research into many of the plant based compounds found within a Mediterranean dietary pattern that potentially act as hormetins and discusses the biological mechanisms through which they are suggested to have their protective effects.

1.6.2 Diet and Osteoarthritis/Pain: Pathological and Epidemiological Evidence

Epidemiological studies alongside intervention studies have focused their attention on diet and anti-inflammatory bioactive compounds. Many dietary compounds have been studied over the years particularly polyphenols: isoflavone, anthocyanin, quercertin, catechin and resveratrol (Rosa, Zulet et al. 2012). It is proposed and there is ever increasing evidence to support the suggestion that a western diet is pro-inflammatory and a plant based diet, seen typically in Mediterranean and African lifestyles, is anti-inflammatory (Dean and Gormsen Hansen 2012). The differences between these two lifestyles are the consumption mainly of meat and a proposed inactive lifestyle in western culture and the Mediterranean diet is largely plant based, rich in fibre and fruit and vegetables and favours olive oil over animal fats (Landaeta-Diaz, Fernandez et al. 2013). This part of the chapter explores dietary compounds and biological mechanisms through which they may confer their beneficial effects.

1.6.2.1 The Importance of Dietary Compounds

Studies have shown that up to 75% of patients believed that their condition is affected by the food they eat. Some foods are thought to exacerbate joint inflammation due to their acidity, actually alkalize the blood and ought to be included in a healthy diet due to being rich in micronutrients e.g citrus fruits which contain vitamin C and tomatoes which contain lycopene. Although randomised controlled trial results reflect amalgamated findings from a group of subjects they could be missing specific needs and therefore benefits on an individual level (Rayman and Pattison 2008).

There has been much research into the effects of different chondroprotective agents on the degradation of cartilage in osteoarthritis. Williams et.al., studied the effect of dietary components on OA, in twins which identified diallyl disulphide (DADs), found in allium vegetables such as garlic to be associated with reduced hip OA occurrence as DADs blocked expression of key matrix-degrading proteases (Williams, Skinner et al. 2010).

There are also reported effects of milk peptides, plant sterol and stanol, l-carnitine and α-lipoic acid on inflammation (Rosa, Zulet et al. 2012). Previous studies have looked at these anti-inflammatory effects using plant agents such as the sterol guggulsterone (found in gum resin) which was found to down-regulate activation of NF-κB, showing potential for the treatment of joint disease (Khanna, Sethi et al. 2007). Intake of plant phytosterols/stanols has been found through intervention trials to significantly reduce lipid levels, such as cholesterol (Wu, Fu et al. 2009). The phytosterol stigmasterol has been found to bind to chondrocyte membranes and inhibit IL-1 induced *MMP* and *ADAMTS4* expression. This was confirmed by the reduction in cartilage degradation due to suppressed MMP expression, through intra articular injection of stigmasterol in rabbits (Chen, Yu et al. 2012).

Previous research has investigated the effects of the Isothiocyanate (ITC) known as Sulforaphane (SFN). ITCs are bioactive compounds found in a number of foods particularly cruciferous vegetables (Steck, Gammon et al. 2007) and are known to interfere with

multiple signalling pathways (Kim, Yeo et al. 2009). There are many structurally related ITCs such as allyl ITC, benzyl ITC, phenethyl ITC and SFN (Tang, Li et al. 2006). Each ITC is formed from the metabolism of a different glucosinolate as seen in Figure 6 (Steck, Gammon et al. 2007), SFN being a good example (Gasper, Al-janobi et al. 2005).

Hydrolysis of the glucosinolates to ITCs is dependent upon a glucohydrolase enzyme known as myrosinase of which activity is from plant enzymes and microflora of the intestines. There are many different glucosinolates found within cruciferous vegetables of which each produces a different ITC. Two of the most abundant glucosinolates in broccoli are glucoraphanin and glucoerucin and when hydrolysed these form SFN and the ITC metabolite erucin (ERN) (Clarke, Hsu et al. 2011). The naturally occurring ITC, SFN, is an inducer of phase 2 enzymes which gives it chemopreventative activity and anti-inflammatory activity has also been identified.

A study aiming to determine whether SFN has any influence over MMP production and its effect on the activation of MAP kinases and NF-κB found that lower concentrations of SFN within chondrocytes were not cytotoxic, but at 50 μM SFN the treatments caused chondrocyte death. Results from this investigation showed that SFN decreased NF-κB protein expression, downregulated NF-κB activation and inhibited MMP production and activation in chondrocytes (Kim, Yeo et al. 2009). This shows success in SFN's ability and potential to become a treatment which has been investigated further by the Clark lab with a SFN feeding trial. This investigation involved participants with end stage OA consuming a high SFN diet for ten days before their joint replacement and it was found that there was a build-up of SFN and that it was indeed measurable within the joint after that ten day period (Davidson, Gardner et al. 2017). Similar results have been reported with ERN bioactivity and it is suggested that a better understanding of the bioavailability of bioactive ITCs is needed (Clarke, Hsu et al. 2011). Investigations into the effect of the bioactive compound SFN on the control of MMP synthesis and activity in chondrocytes has demonstrated it to be a chondroprotective agent and it is being targeted in an attempt to inhibit degeneration of cartilage in OA (Kim, Yeo et al. 2009).

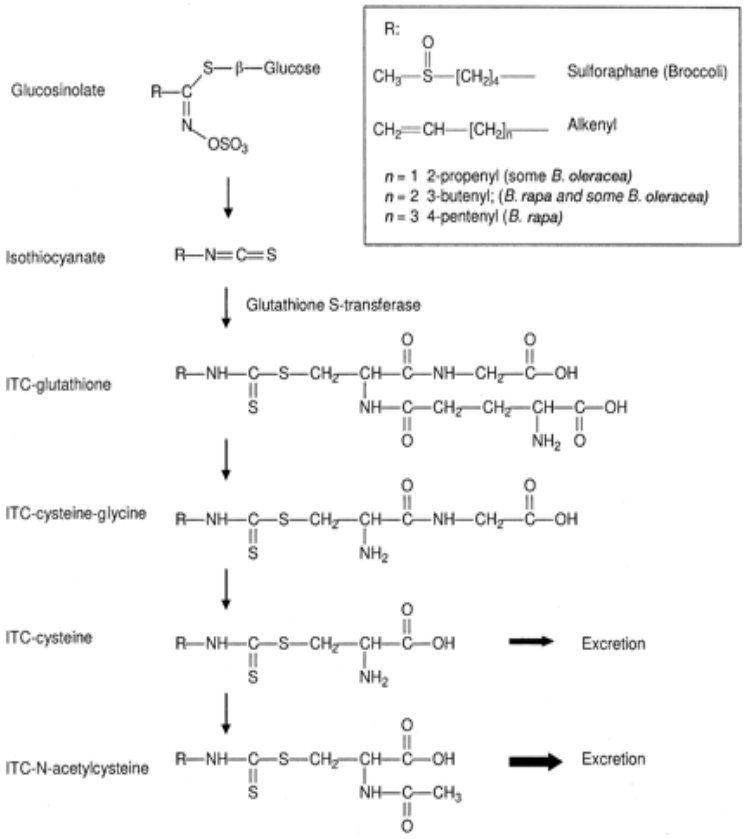

FIGURE 7. THE METABOLITES OF ITCS (GASPER, AL-JANOBI ET AL. 2005).

Research into the chondroprotective effects of pomegranate extract have revealed cartilage-protective active compounds as well as green tea which has also been shown to have anti-arthritic effects (Khalifé and Zafarullah 2011). This is due to the catechins, also found in dark chocolate, which have been found to inhibit cartilage degradation in vivo (Adcocks, Collin et al. 2002). Epigallocatechin gallate and epicatechin gallate have also been shown to effectively inhibit ADAMTS-4 and -5 activity (Vankemmelbeke, Jones et al. 2003).

Berries, containing anthocyanins (Manach, Scalbert et al. 2004), curcumin found in turmeric and resveratrol, found in the skin of red grapes, have all shown effects in improving pain and inflammation in OA.

A recent study that investigated the effects of strawberries on pain and markers of inflammation found that in obese adults with radiographic knee OA, IL-1, IL-6 and MMP3 were significantly decreased and pain was also reduced after 12 weeks of daily strawberry supplementation (Schell, Scofield et al. 2017). Resveratrol is an activator of Sirt1 (Lam, Peterson et al. 2013) which when deleted in mice causes an increased rate of development of OA (Matsuzaki, Matsushita et al. 2013). Resveratrol has been found to inhibit NF-κB in chondrocytes and is able to block inflammation and apoptosis (Lei, Wang et al. 2012). Resveratrol has also been found to have synergistic effects on chondrocytes with curcumin (Shakibaei, Mobasheri et al. 2011). Curcumin has been found to be an inhibitor of NF-κB, JNK, STAT and MAPK signalling, inhibiting MMP expression in cartilage (Innes, Fuller et al. 2003), COX2 expression and other inflammatory mediators (Shakibaei, John et al. 2007).

1.6.2.2 Micronutrients

Epidemiological studies have looked at a variety of components within the diet in relation to radiographic OA. A study using the Hertfordshire Cohort, which looked at Vitamin D intake and Vitamin D Receptor Polymorphisms, showed Vitamin D intake to be associated with knee pain (Muraki, Dennison et al. 2011). It is suggested that Vitamin D has a direct effect on cartilage metabolism as it is believed to be a stimulant for the synthesis of proteoglycan in mature chondrocytes (Corvol, Dumontier et al. 1981). A Framingham Cohort Study revealed an increased risk of the progression of OA with lower Vitamin D intake (McAlindon, Felson et al. 1996) and a reduction in risk for radiographic OA with a higher consumption of Vitamin C (McAlindon, Jacques et al. 1996).

Vitamin C is considered a highly effective antioxidant due to its ability to react with free radicals and ROS (Li, Zeng et al. 2016) and has been shown to prevent changes such as increased oxidative stress, loss of proteoglycan, apoptosis. It has been found to prevent increased levels of pro-inflammatory cytokines such as ILs, MMPs and TNF-α induced in an in vitro model using chondrocyte-like cells (Chiu, Hu et al. 2016). A reduction in the risk of knee OA in terms of reduced cartilage loss has been found with Vitamin C intake as well as reduced risk of the development of knee pain (McAlindon, Jacques et al. 1996) although more recently there have been some conflicting results from cross-sectional studies (Li, Zeng et al. 2016). Longitudinal studies have not found Vitamin C to be protective of progression of knee OA however multivariate analyses has shown that Vitamin C supplements have a protective effect on the development of knee OA (Peregoy and Wilder 2011).

Beta carotene, a precursor to Vitamin A (Maiani, Periago Castón et al. 2009), and Vitamin E were also seen to reduce the risk of OA progression (McAlindon, Jacques et al. 1996). Intake of other carotenoids, such as lutein and zeaxanthin, are associated with decreased risk of cartilage defects (Wang, Hodge et al. 2007) and Vitamin E is suggested to protect against changes in ECM gene expression when induced by hydrogen peroxide (Mehmood, Wajid et al. 2013).

The Research on Osteoarthritis against Disability (ROAD) study was designed in 2005 in order to clarify both genetic and environmental risk factors of OA. Dietary nutritional factors and the prevalence of radiographic knee OA were investigated and Vitamin K intake was lower in those groups that had a higher Kellgren Lawrence grade suggesting that Vitamin K, which regulates mineralisation in bone and cartilage (Krüger, Westenfeld et al. 2009), may have a protective role in OA (Oka, Akune et al. 2009). A study in Japan found that Vitamins K, B1, B2, B6 and C showed an association with minimum joint space width and Vitamins K, E, B1, B2, B3 (niacin) and B6 showed an association with osteophytes in females only (Muraki, Akune et al. 2014).

1.6.2.3 Dietary Fatty Acids

Fatty acids are essential nutrients and are involved in a number of biological processes. Fatty acids within the diet consist of saturated fats, trans-fatty acids, mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs) (Tokuyama and Nakamoto 2011).

As previously mentioned obesity is a major risk factor for OA (Elliott, Chapman et al. 2013) and excess weight can be due to diet and the saturated fats being incorporated into the adipose tissue along with MUFAs which can either be stored or oxidized for energy with the unsaturated fats (Lopez 2012).

The main dietary polyunsaturated fatty acids (PUFAs) can be classified as either long chain ω-3 (n-3) or functional ω-6 (n-6). ω-3 Fatty acids (FAs) e.g. eicosapentaenoic acid (EPA) and α-linolenic acid (LA) are found in dietary sources such as fish oils, walnuts and canola oils whereas ω-6 FAs e.g. LA and arachidonic acid (AA) can be found in foods such as corn, soybean, sunflower oils and meats (Brenna, Salem et al. 2009).

Fatty acids are suggested to be of great importance as palmitic, oleic and linoleic acids account for 85% of total fatty acid content in cartilage (Villalvilla, Gómez et al. 2013). The most abundant MUFA in the human diet is the ω-9 FA Oleic acid. Some MUFAs and saturated FAs can be synthesized from proteins and carbohydrates within the diet but the enzymes needed to form ω-3 or ω-6 FAs are lacking, ω-3 ALA and ω-6 LA cannot be produced and converting ALA into EPA and Docosahexaenoic acid (DHA) is limited in humans. Those who suffer from metabolic syndrome, iron, vitamin or mineral deficiencies, consume ω-6 FAs, trans-FAs and alcohol in excess or are on medications that are known to affect metabolism have decreased production of EPA and DHA metabolites from ALA (Brenna, Salem et al. 2009).

Eicosanoids and docosanoids are the end products of the cyclooxygenase and lipooxygenase pathways (Lopez 2012) (which synthesize prostaglandins and leukotrienes (Villalvilla, Gómez et al. 2013)) as a result of the fatty acids EPA and DHA being utilised as a substrate and are known to have anti-inflammatory properties. However when the ω-6 FAs LA and AA are used, the end products instead have pro-inflammatory properties. ω-6 FAs, although important in other biological processes, when taken in excess levels to ω-3 FAs seem to shift the eicosanoid profile towards one that is pro-inflammatory (Lopez 2012). Unsurprisingly inhibition of cyclooxygenase has been found to lead to less inflammatory mediators (Villalvilla, Gómez et al. 2013). EPA and DHA are also very beneficial for cardiovascular, immune, cognitive and metabolic health alongside a positive contribution to muscular strength and function, mood and vision when ω-3 fish oil supplements rich in these PUFAs are consumed.

Dietary lipids are thought to play an important role in carotenoid bioavailability despite their low lipids levels (Unlu, Bohn et al. 2005). De Roos *et.al.,* conducted a study that found individuals, with serum levels of trans-beta-carotene and zeaxanthin were more likely to have knee OA whereas serum levels of the carotenoids lutein or beta-cryptoxanthin were 70% less likely to have knee OA than the controls (De Roos, Arab et al. 2001).

Saturated fatty acids and MUFAs are used as substrates for energy however PUFAs are metabolised to produce phospholipids and prostaglandins which have a number of biological functions. Dietary deficiencies in ω-6 LA and ω-3 ALA have been found to result in a number of different health problems (e.g. lowered immunity) showing their vital role (Tokuyama and Nakamoto 2011) which has earned them the term essential fatty acids as the human body can synthesize all but these two of the fatty acids it requires (Patterson, Wall et al. 2012). Human studies investigating the effect of the different fatty acids types on OA are limited to knee OA and are inconsistent (Loef, Ioan-Facsinay et al. 2019). The

figure below shows the metabolism of PUFAs within the diet (Tokuyama and Nakamoto 2011).

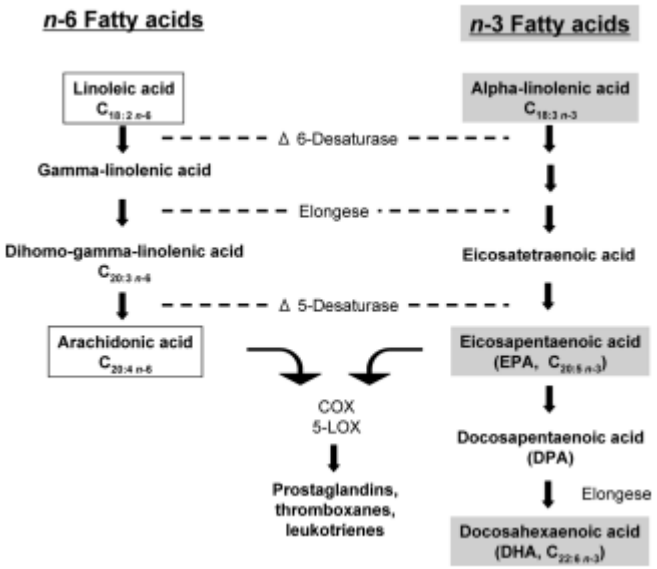

FIGURE 8. PUFAS AS PRECURSERS FOR COMPLEX AND INTERLINKED PATHWAYS OF METABOLISM (TOKUYAMA AND NAKAMOTO 2011).

1.6.3 Availability and Sources of Fatty Acids and Lipids

Studies in the 1960s found that 0.5%-1% of human cartilage was made up of lipids (Stockwell 1967). Since then many studies have investigated lipid metabolism in relation to rheumatic signs and symptoms due to there being a link between inflammation, tissue repair, defence mechanisms and fluctuations in lipid levels (Esteve, Ricart et al. 2005). The status of cartilage and the general development of OA is known to be dependent upon the availability of lipids (Villalvilla, Gómez et al. 2013) and lipoproteins are suggested to play a crucial role in the process of acute and chronic inflammation (Oliviero, Nigro et al. 2012).

As important nutrients in chondrocyte structure, signalling and metabolism, lipids are available to chondrocytes, which make up 2%-5% of total cartilage, through diffusion from surrounding tissues, e.g. synovial fluid, although availability is restricted by matrix permeability dependent upon the charge and size of molecules and also through de novo synthesis. Phospholipids are found within the cell membrane and the synovial fluid and are thought to play a key role in lubrication to protect cartilage surface.

Phosphatidylethanolamine, phosphatidylcholine and sphingomyelin are all major components of cartilage boundary lubricant and free fatty acids may be incorporated by chondrocytes into these components as well as phosphatidylinositol and triacylglycerol. Extracellular fatty acids have been found to control lipid composition in chondrocytes and changes in lipid composition in cartilage have been found to be correlated with severity. In the advanced stages of OA there have been increases seen in total fatty acid content of as much as 440%. Both cholesterol biosynthesis and fatty acid metabolism occur in normal chondrocytes and lipids are required to travel through the complex matrix within cartilage. Fatty acids penetrate the ECM much more easily than larger complexes and research is

trying to understand exactly how this happens and how lipids then affect chondrocyte metabolism (Villalvilla, Gómez et al. 2013).

Despite mature joints containing calcified cartilage which creates a potential barrier to lipid diffusion between calcified and non-calcified cartilage (Villalvilla, Gómez et al. 2013), both mature and immature joints are alike in their cartilage permeability for uncharged solutes (Torzilli, Grande et al. 1998). The matrix is negatively charged which stops movement of smaller negatively charged molecules but does allow movement of smaller positively charged solutes which means ability to move through cartilage depends on valency and molecule affinity for the matrix and larger molecules have altered movement through due to their charge (Maroudas 1976). In order to increase their solubility lipids exist as lipoproteins in blood plasma or bind to carrier proteins but becoming a larger molecule does then restrict their movement through cartilage. However, some larger molecules are able to separate in order to travel through tissues e.g. lauric acid separating from albumin at the surface of cartilage so that both can independently diffuse through (Arkill and Winlove 2006).

It has been found that dietary lipid intake can affect fatty acid composition in cartilage (Xu, Watkins et al. 1994). Cartilage permeability increases with the progression of OA due to the increase in proteases (LOTKE and GRANDA 1971). Lipid movement through cartilage can, therefore, be easily achieved by diffusion from synovial fluid (Maroudas 1976) which contains proteins that can reach chondrocytes and initiate an inflammatory response (Sohn, Sokolove et al. 2012). Diffusion of molecules from the synovial fluid can be modified by repeated loading and compression (Villalvilla, Gómez et al. 2013) and due to the increased synovial permeability under inflammatory conditions and the ability of synovial fluid to filter plasma, lipoproteins are suggested to be able to diffuse from the circulation into the synovial fluid. Again this is thought to be determined by inflammation and vascular permeability of the synovium as well as the plasma concentrations of each lipid. In inflammatory arthritis, lipid levels have been found to be higher in synovial fluid except for HDL. The majority of serum lipid levels have been found to be higher specifically in OA as opposed to other inflammatory arthritides and fluctuations in these lipid levels are mediated by cytokines (Oliviero, Nigro et al. 2012) .

Previous studies have also investigated whether subchondral bone also has the ability to provide lipids to cartilage. The transport of molecules from subchondral bone to cartilage in mice was found and suggested to be due to pores in non-mineralised regions of calcified cartilage allowing solute transport (Pan, Zhou et al. 2009). There have been suggestions of this same mechanism in humans but molecular transport has since been suggested to exist between bone and bone marrow vessels and calcified cartilage through the overlapping extensions of cartilage within the chondro-osseous region of mature cartilage (Lyons, McClure et al. 2006).

The evidence throughout this chapter highlights the differences between dietary fatty acids and confirms the sources and role of lipids in OA. Lipid accumulation in cartilage, as a result of diet however, still needs clarification (Xu, Watkins et al. 1994).

1.7 Obesity and Osteoarthritis

1.7.1 Overnutrition

Obesity and over eating is an existing problem which can lead to a variety of health issues and nutrition, when in excess, can bring on lipotoxic effects (Pottie, Presle et al. 2006). There is now a well-established link between obesity and OA however, the story of the role of excessive body weight in the development of OA is ongoing.

Previously there have been many epidemiological studies that have investigated the effects of body mass on the main weight baring joints; the hip and the knee but with other joints such as the hand also at risk of OA comes the suggestion that obesity does not just have direct effects in terms of more weight loading on joints (Pottie, Presle et al. 2006) but that other biological mechanisms could be at work (Triantaphyllidou, Kalyvioti et al. 2013) there seems to be evidence for the existence of complex metabolic pathways in the pathology of rheumatic disease (Pottie, Presle et al. 2006). Studies have more recently found OA to be influenced by dietary fatty acid content (Wu, Jain et al. 2014). A study on obese patients with chronic OA using a 12 week weight reduction program revealed that a diet low in calories, with a commercial formula, was able to improve body weight, body fat, blood lipids and metabolic response. In addition patients with greater than 9% weight reduction reported a better quality of life (De Luis, Izaola et al. 2012).

Changes in lipid metabolism are a suggested influence on OA (Triantaphyllidou, Kalyvioti et al. 2013) but the question remains as to whether this is a potential risk factor or a consequence of OA (Villalvilla, Gómez et al. 2013). This chapter will continue to discuss lipids, lipid metabolism and fatty acid associations with OA.

1.7.2 Obesity and Hand OA

“Obese people do not walk on their hands” (Yusuf 2012). There is a definite link between obesity and the development of OA: a risk factor that has long been recognised (Felson, Lawrence et al. 2000). BMI has been found, in the Rotterdam Study, to be associated with osteoarthritis of the DIP, PIP and metacarpophalangeal joints in the hand (Hoeven, Kavousi et al. 2013). Osteoarthritis of the hip and knee can be attributed to the overloading of or increased stress as they are weight baring joints but occurrence of OA in the hand, a non-weight baring joint, cannot be explained by an increase in weight. It is quite clear, therefore that other factors play a role in the development of hand OA.

Osteoarthritis has been linked to fluctuations in hormones for over a century. Terms such as ‘menopausal arthritis’, ‘climacteric arthritis’, ‘endocrine arthritis’, ‘hypoglandular arthritis’, ‘chronic villous arthritis’ and ‘primary generalised OA’ have all been used over time, since the 1920s whilst studies continued to try and explain the stiffness, inflammation, pain and the presence of Heberden’s nodes in the joints of those that were typically middle aged, obese and female (Spector and Campion 1989). One of the earlier studies in 1938 noted that some of these joint symptoms occurred post menopause or after having a hysterectomy (Hall 1938). Later in the 1980s, a case control study found that women with OA had twice the number of hysterectomies in comparison with the age matched controls (Spector, Brown et al. 1988). A more recent study found that joint

symptoms are twice as likely to be seen in post-menopausal women as in those experiencing menopause as shown by findings from the Melbourne Women's Mid-life Health Project. Hand OA, when experienced post menopause, also frequently occurs without OA occurring at other sites (Szoeke, Cicuttini et al. 2008).

Previous observational studies of HRT and hand OA have shown mixed results. A study in 1997 found that current MHT use showed a protective effect for distal interphalangeal (DIP) joint OA (Spector, Nandra et al. 1997), however a study in 2003 then reported that use of HRT was associated with more severe and a higher prevalence of distal interphalangeal (DIP) disease (Cooley, Stankovich et al. 2003). There have been no differences found in MHT use or non-use and hand OA (Maheu, Dreiser et al. 2000), however, there has been a positive association found between hand OA and MHT when taken for less than 5 years and those using MHT for more than 1 year were also found to be at an increased risk of hand OA (Von Mühlen, Morton et al. 2002).

Studies investigating associations between reproductive factors and cartilage have found no association with HRT (Wei, Venn et al. 2011). Obesity in females is a cause of hyperoestrogenism due to the formation of oestrogen from androstenedione in adipose tissue suggesting the endocrine system plays a key role in OA development. However, it was once thought that a lack of oestrogens could be a cause of OA but after years of studies showing that oestrogens do not improve clinical OA it has since been suggested that the ratio of high concentrations of oestrogen, found both before and after menopause, in relation to the decreasing levels of progesterone is of more importance with regard to the development of OA (Spector and Campion 1989).

1.8 The Role of Genetics in the development of OA

In addition to biomechanical and inflammatory mechanisms, genetics are also suggested to play a role in the development of OA/pain. Dietary modification to reduce occurrence of disease is now being investigated widely but this can be difficult due to the complex genetic factors that influence food choice. The role of nutritional factors in OA needs to be interpreted against the knowledge that there is a genetic contribution to OA. With around half of the risk of developing clinical OA accounted for by heritability (Zengini, Finan et al. 2016), this chapter explores the strong genetic component in OA.

The finding of genes that influence OA have come to light through genome wide association studies (GWAS). GWAS has widely recognised many SNPs which means studies can now investigate those genetic factors most important in the risk of OA (Hochberg, Yerges-Armstrong et al. 2013). Due to SNPs occurring randomly every 100 to 300 nucleotides throughout the genome and the discovery that SNP sequences at specific loci are shared by the population, SNPs have been used as a genetic testing marker (Klug, Cummings et al. 2009). Studies are now identifying genes involved in the development of OA which could provide clues as to the pathological progression (Hochberg, Yerges-Armstrong et al. 2013).

There have been various candidate genes, found to be implicated in OA. The arcOGEN GWAS, which was a large powered GWAS, found that the genes type II procollagen (*COL2A1*), oestrogen receptor-α (*ERS-α*), growth differentiation factor 5 (*GDF5*), double von Willebrand factor A domains (*DVWA*), fizzled-related protein (*FRZB*), *Asporin* and *IL-6* all

conferred susceptibility to OA (Consortium 2012). Due to few genetic variants having been identified for OA, a more recent GWAS investigating a structural component of OA to try and reduce heterogeneity identified four novel loci phosphatidylinositol 3-kinase receptor 1 (*PIK3R1*), fibroblast growth factor receptor 3 (*FGFR3*), trehalase (*TREH*) and transforming growth factor α (*TGFA*) to be associated with cartilage thickness (Castaño-Betancourt, Evans et al. 2016).

Of particular interest are those genetic factors that have a shared aetiology with obesity and lipid metabolism. By gaining access to genes of interest, investigations into the mechanisms of cause and effect, by Mendelian Randomisation, will allow us to see whether a particular risk factor that contributes to OA and pain is indeed causal. This will contribute to the current knowledge of genetic factors and their influence on OA and will also help us to understand the relationship that exists between diet and OA.

Variants of the fat mass and obesity associated gene *FTO*, are of particular interest as it is known to be associated with increased risk of obesity. Variants of *FTO* are suggested to affect the functioning of adipocytes by disrupting repression of Iroquois 3 (*IRX3*) and Iroquois 5 (*IRX5*) causing a shift from energy dispelling brown adipose tissue to energy storing white adipose tissue (Claussnitzer, Dankel et al. 2015). The SNP rs1421085, found within the fat mass and obesity associated gene, is known to be associated with increased risk of obesity due to a T to C change. Those with the genotype that is homozygous for the risk allele are, on average, 3 kg heavier than those with the genotype that is homozygous for the protective allele (Frayling, Timpson et al. 2007). Both rs1421085 and rs17817449, found within *FTO*, have been found to be associated with adiposity, waist circumference, hip girth, body fat and weight (Do, Bailey et al. 2008) The SNP rs8044769 was also found among hundreds of others to be associated with obesity and has been reported to affect other SNPs from *FTO* (Frayling, Timpson et al. 2007).

The Apolipoprotein A5 (*APOA5*) gene is linked to lipid metabolism (Chen, Ding et al. 2018). The *ApoA1/C3/A4/A5* gene cluster is a widely investigated and well characterised region of the genome found to be associated with lipid levels (Gombojav, Lee et al. 2015). It has also been suggested that differences seen in serum lipid levels could be due to interactions between *APOA1/C3/A5* haplotypes and alcohol consumption (Yin, Li et al. 2013). With triglycerides, total cholesterol, LDL-C and HDL-C not only being heritable risk factors but also being targets for intervention of disease (Teslovich, Musunuru et al. 2010) genetics studies investigating genes associated with lipids and obesity are becoming increasingly popular and important.

Studies investigating the genetics of lipid levels have been focused mostly within European cohorts but there is potential to identify novel genes if studies could investigate lipid levels in other cohorts. For instance, using the EpiDREAM and INTERHEART cohorts. A study using these cohorts scanned 31, 739 common SNPs from candidate genes to try and identify novel genetic modifiers of plasma ApoB levels and found a significant association between SNP rs4664443, within dipeptidyl peptidase-4 (*DPP4*), and cholesterol, LDL-C and also ApoB but only in those with a BMI <25 kg/m². Another study identified just under 100 loci in GWAS data that showed significant genome wide associations with circulating lipids. (Teslovich, Musunuru et al. 2010). Looking at lipids specifically between genders, due to the suggested differences that exist in heritability between males and females (Weiss, Pan et al. 2006), four of the ninety-five loci found showed significant differences between men and women and five additional loci showed significant associations in females. SNP

rs1562398, from Krüppel-like factor 14 (*KLF14*), for instance was found to be associated with HDL-C in both genders but was significantly associated with triglycerides just in females. ATP-binding cassette subfamily A 8 (*ABCA8*) was also found to be associated with HDL-C in both genders and was associated with triglycerides and LDL-C but again just in females.

DNA variants have also been found to regulate expression levels of nearby genes in liver and fat tissues. For example SNP rs9987289 (associated with both LDL-C and HDL-C) was found to be associated with changes in liver expression of protein phosphatase 1, regulatory (inhibitor) subunit 3B (*PPP1R3B*) (despite being 174kb away from the gene). SNP rs2972146 (associated with HDL-C and triglycerides) is correlated with gene expression of insulin receptor substrate protein 1 (*IRS1*) in omental fat, but is found 495kb away from the gene (Teslovich, Musunuru et al. 2010).

Whilst GWAS studies revealed 18 loci associated with OA, this only accounts for a very small proportion of the genetic contribution in OA. Therefore, more research is needed to investigate interactions and contribute to current knowledge, in the hope that underlying mechanisms between genotype and phenotype can be better understood and potentially lead to other approaches to treating OA (Zengini, Finan et al. 2016). SNPs are, therefore, of great importance and can be used as instrumental variables in Mendelian Randomisation studies to investigate reverse causality.

It is also worth noting here that epigenetic alterations may be playing a role in the genetic component of OA. Instead of a genetic mutation and therefore a specific allele directly affecting the risk of OA it could be that heritable modifications in gene function (which occur quickly and in response to environmental changes without altering the underlying DNA sequence) could be contributing towards the development of OA (Barter, Bui et al. 2012).

1.9 Summary of the Contribution of Nutrition and Rationale for the Present Study

Despite the various biomechanical, genetic, endocrine and inflammatory factors that influence osteoarthritis and pain, the eitopathogenesis of OA still needs clarification (Pavelka 2017). Although there are many of studies contributing to the knowledge of diet and nutrition with a focus specifically on OA and associated symptoms, further investigation is needed and replication and validation of results in other datasets should also be conducted to confirm findings (Deutsch 2007). More research into this area could result in lifestyle improvements and better health and wellbeing opposed to a lifestyle of surgery, drugs, interventions and side effects. Studies are needed to investigate those who respond to nutrition and those who do not. Future studies also need to assess the risk factors, at the rates at which they are reduced, in those whose primary treatment is dietary intervention (Dean and Gormsen Hansen 2012). By promoting healthy nutrition, less saturated and trans-fat content would exist in the diet, which is the suggested mechanism by which inflammation is caused through being overweight (Liu, Wei et al. 2013). By changing lifestyle factors, such as nutrition, as a way of treating inflammation and alleviating symptoms there is potential for reducing the rate of the development and progression of OA.

Many types of studies exist, from cell culture to identify compounds of interest and laboratory studies to improve our understanding of the mechanisms of action to metabolic studies which look into the physiologic effects. However epidemiologic studies are needed to look at the relationship between diet and disease directly (Willett 2012). Further research into diet and nutrition and the finding of potential associations could lead to the identification of certain aspects of diet that may be able to alleviate symptoms or slow the progression of OA. It may also provide information before onset and therefore reveal a dietary prevention strategy easily implemented on a population scale. New evidence (although limited) is emerging through the literature regarding nutrition and OA but these effects and the relationship between nutrition and OA/pain need clarifying. Further research is needed before there is certainty about specific associations seen between diet and OA and the direction of causality between these associations.

Research investigating lifestyle factors has improved over recent decades as large population based data resources have become available. These include cohorts such as the European Prospective Investigation into Cancer (EPIC), TWINS-UK, the Rotterdam Study, the Chingford Study and the Osteoarthritis Initiative (OAI). Detailed phenotypic information on the clinical features of OA and OA pain such as radiographic data and clinical symptoms together with extensive data on dietary exposures, relevant lifestyle factors such as smoking and physical activity and genetic data from GWAS exist. These large population data sets act as very useful resources for investigating OA. They are sufficiently powered due to their large numbers and are prospective cohort studies which identify exposure at present and determine future disease onset as opposed to being retrospective which arguably are quicker and more cost efficient but relies on sufficient information being available on past exposure, current exposure and risk factors. Therefore prospective cohort studies are considered the most powerful methodology as limitations such as recall bias etc. do not affect the cohort and can also account for changes to exposure status (Silman and Macfarlane 2002). These studies are also of longitudinal design with follow-up of participants and collection of additional data allowing further study of disease over multiple time points. This produces more reliable results than cross-sectional analyses where data is investigated at a single point in time.

With these cohorts, research into diet and its effects on OA and pain can take place, increasing our knowledge of the contribution of various risk factors towards OA. In particular through investigating SNPs of interest, helping to identify those more at risk and leading to new ideas for treatment (preventative or otherwise). With OA causing dramatic changes to lifestyle, leading to other health problems and with numbers in the population showing it to be on the increase there is a need to investigate potential ways in which patients may be able to change aspects of their lifestyle in order to alleviate their symptoms successfully.

In summary diet and nutrition is able to influence the underlying mechanisms such as inflammation involved in OA and research is ongoing into finding those dietary components that could have pro or anti-inflammatory effects. However more research is needed specifically into the associations that exist between nutrition and OA/pain for which sufficient dietary data is needed. Two of the large cohorts above, TWINS-UK and a subset of the EPIC cohort (EPIC-Norfolk cohort) are used in this research to provide additional insight into those lifestyle and dietary factors that play a role in OA and the onset of pain.

There are numerous studies on the Mediterranean diet and it's suggested protective association with chronic diseases that are underlined by inflammaging. However, as discussed in Chapter 1, there are a very limited number of studies looking at associations between Mediterranean diet and OA specifically. Most of these studies have focused on knee OA and have also been of cross-sectional design so further studies are needed to investigate associations between dietary factors and OA particularly of other joints, especially non weight bearing joints such as the hand. The effects of dietary factors on pain have also been investigated previously but there is limited research looking at the effects of diet on pain specifically associated with OA. The TWINS-UK cohort is a great resource and provides a large volume of dietary data, from which a Mediterranean dietary pattern can be created, as well as radiographic OA and musculoskeletal pain data. This will allow an analysis of the analysis of dietary and OA/pain variables in order to further the knowledge of how diet as a modifiable factor may influence the onset or progression of OA and associated pain. In addition this research provides an opportunity for associations to be looked at in greater detail through analysis of genetic data from EPIC which allows for causal mechanisms to be investigated holding the potential to confirm the direction of causality between associations observed.

2 Study Design and Aims.

This chapter discusses the collection and use of the data, why these two cohorts are suited to this research and the study design and statistical methods used to investigate the effects of lifestyle and dietary factors on OA/pain.

2.1 Cohorts

2.1.1 TWINS-UK Data

With continuing genetic studies, the demand for twin cohorts has increased and resulted in twin registries forming around the world. Cohorts such as the Danish Registry, the Finish Twin Cohort Study and the UK Adult Twin Registry (TWINS-UK) all contain thousands of phenotyped twins and hold this data for a number of different traits (Moayyeri, Hammond et al. 2013). The present study uses data collected by the UK Adult Twin registry which was started in 1993. The cohort of approximately 10,000 twins, aged 16 to 85, were recruited through media campaigns with an overall aim of recruiting healthy volunteers and not selecting individuals for specific studies. Most twins are subjected to clinical assessment and given detailed questionnaires regarding aspects of lifestyle at many different time points (Spector and Williams 2006). All data was collected at St. Thomas's Hospital, Kings College London, and passed onto researchers at the Norwich Medical School, University of East Anglia in a collaborative effort to investigate the associations between nutrition, genetics and disease. Data was cleaned eliminating those individuals that have missing data for the variables of interest. Datasets were then merged, using Stata, in order for the variables for each individual of interest to be contained within the same dataset allowing statistical analysis to take place.

The dietary data, used in this study, consists of dietary food patterns. Dietary data was collected through food frequency questionnaires (FFQs) and principle component analysis (PCA) created five dietary derived patterns (discussed further in Chapter 6). This type of data collection enables the capturing of data over a long period of time in order to investigate associations between dietary intake and disease (Willett 2012). FFQs are known to be sufficient at measuring dietary intake, however, due to inaccuracies in estimating food consumption frequency, questionnaires are still not as reliable or accurate as weighed food diaries (Bingham, Gill et al. 1994). One of the main reasons for the inaccuracy of FFQs is due to the nature of under-reporting by participants. The FFQ used in the Twins was the same one that had been previously used in EPIC (discussed further in Chapter 6) which was validated against urinary biomarkers and plasma ascorbic acid levels (Bingham, Gill et al. 1997).

2.1.2 EPIC and EPIC-Norfolk Data

The European Prospective Investigation into Cancer (EPIC) study contains half a million adult volunteers recruited between 1992 and 2000 and is a multicentre prospective study with participants from 23 centres across 10 western European countries. The study was designed primarily to follow participants for cancer incidence and cause-specific mortality

but has been used to study other diseases as well. The ages ranged between 35 and 70 years and the study populations are samples of volunteers agreeing to participate.

The data used in this study, from the EPIC-Norfolk cohort, is an example of a prospective cohort study whereby exposure in the past is taken into account and disease status currently is investigated. It contains clinical OA data from the Hospital Episode Statistics (HES) Database, joint pain data and dietary data (outlined further in Chapter 3).

Questionnaires used included questions on education, socio-economic status, current job, current and past occupation which might have led to exposure to carcinogens, history of previous illness, disorders or surgical operations, lifetime history of tobacco smoking, lifetime history of consumption of alcoholic beverages, physical activity (occupational, walking, cycling, gardening, housework, physical exercise, climbing stairs), menstrual and reproductive history and use of exogenous hormones for contraception and postmenopausal replacement therapy.

Those adult volunteers who gave informed consent were invited to participate either in person or by post. Firstly individuals were sent a questionnaire on diet and a questionnaire on lifestyle which, for most, were completed at home and a visit to the study centre for an examination followed. Lifestyle variables were collected through non dietary questionnaires and separate dietary questionnaires addressed usual diet. Blood samples were taken from so called healthy populations from which fractions were separated and stored long-term. Nutritional methods in EPIC-Norfolk included FFQs, 7 day food diaries and standardised computer-assisted 24-hour dietary recall which took place at each centre on stratified random samples to calibrate dietary measurements, making the sub-cohorts comparable on an absolute scale, correcting for over and underestimations in baseline dietary assessments between centres. The study follows the participants in order to pick up on the occurrence of cancer, disease and to observe overall mortality. This allows for incidence and mortality to be compared by exposure variables. For any changes to aspects of lifestyle that are strongly suspected or well known to be related to disease risk, follow-up questionnaires are used at regular intervals to keep information up to date (Arriola, Martinez-Camblor et al. 2010).

2.2 Study Design and Statistical Analyses

Epidemiological studies are needed to investigate factors which affect the existence of a health problem in the hope of gaining control over disease (Domínguez-Almendros, Benítez-Parejo et al. 2011) and, in the case of the present study, help to establish potential causes of clinical and radiographic OA. The aim of this epidemiological study is to use previously validated statistical methods to predict and assess the onset and development of OA and pain in OA in relation to dietary intake.

Cohort studies, when conducting longitudinal analysis, allow for the control of confounding effects and participants are followed to investigate disease rates whilst analysing dietary data collected from participants who do not have the disease. Cohort studies are also less susceptible to bias as disease cannot affect diet due to dietary data being collected before onset. With larger numbers in these cohorts the relationships between diet and disease are not affected by low rates of participation. These studies also allow for repeated assessment over time which has been made possible by use of self-administered questionnaires.

However the disadvantages are that this is quite costly and there can be losses in follow up data. Some cohorts do not have a sufficient number of cases, therefore, case-control studies are a useful study design used in nutritional epidemiology (Willett 2012).

Using the cohorts described previously containing information about current exposure and disease state, there is the chance to determine the association of potential risk factors with the disease. The EPIC-Norfolk cohort is utilised in this research but using a case-control study design specifically for Chapters 3, 4 and 9 as this study design allows results to be produced more quickly due to participants not being followed, however, the validity of results must be considered in relation to potential methodological bias. This study design is also inexpensive, in comparison to other study designs, whilst identifying those with chronic cases whether symptoms are short lived or long term.

EPIC-Norfolk analysis in this thesis is of both a cross-sectional and longitudinal design whereas TWINS-UK analysese are cross-sectional only. A longitudinal study design can be used in this cohort study to follow subjects over time. These studies aim to determine whether the status of initial exposure might subsequently influence the risk of disease. Within the cross-sectional design of this research is also a type of association study. These are used when there are many measures of association needed to quantify the level of increased risk of exposure to predictor/explanatory variables which could be responsible for the onset of disease (Silman and Macfarlane 2002). In this study odd ratios are the effect measure used to quantify the strength of associations in both the cross-sectional and longitudinal study design used within this research.

EPIC-Norfolk is used in this research to identify factors that best predict OA and pain, a case-control analysis using a logistic regression method, with a backwards stepwise algorithm, removed each variable that is non-significant to leave those variables most significantly predicting the outcome (Hip OA, Knee OA, Hip pain and Knee pain) as those that best explain the variation in the data. This method was chosen due to the modest number of selected potential explanatory variables in EPIC-Norfolk. In case-control studies, data relating to potential risk factors is collected from patients already with the disease and compared to those in which the disease is absent.

Within the TWINS-UK cross-sectional analysis a multi-level mixed effects logistic regression with adjustment for confounders of the diet-OA/pain relationship is used. This method was chosen as it takes into account the hierarchical structure within the data (that participants exist as individuals but within twin pairs). A logistic regression analysis is able to measure the strength of a relationship between two variables of interest; the dependent variable being radiographic OA and musculoskeletal pain which is assessed in relation to the independent variables (dietary pattern scores) whilst allowing for control over confounding factors. This is one of the most widely used methods in epidemiological research (Domínguez-Almendros, Benítez-Parejo et al. 2011).

Table 1 describes the data, study design and statistical analysis for each of the following analysis chapters.

TABLE 1. OVERVIEW OF THESIS ANALYSIS CHAPTERS.

Analysis Chapters (Chapters 3-10)	Cohort	Data	Study Design	Statistical Analysis
Chapter 3. An Investigation into associations between Dietary and Lifestyle Factors and Osteoarthritis/Pain of the Hip and Knee.	EPIC-Norfolk	Cross-sectional	Case-control Study	Regression analysis with Stepwise Algorithm and Randomisation Function.
Chapter 4. A Gender-Specific Investigation into Dietary and Lifestyle Factors and Osteoarthritis/Pain of the Hip and Knee.	EPIC-Norfolk	Cross-sectional	Case-control Study	Regression analysis with Stepwise Algorithm and Randomisation Function.
Chapter 5. Exploring Dietary Patterns and Radiographic OA of the Hip, Knee and Hand.	TWINS-UK	Cross-sectional	Cohort Study	Multi-level Mixed Effects Logistic Regression
Chapter 6. Exploring Dietary Patterns and Musculoskeletal Pain/Chronic Widespread Pain.	TWINS-UK	Cross-sectional	Cohort Study	Multi-level Mixed Effects Logistic Regression
Chapter 7. Exploring the Mediterranean Dietary Pattern and Radiographic OA, Musculoskeletal Pain and CWP.	TWINS-UK	Cross-sectional	Cohort Study	Multi-level Mixed Effects Logistic Regression
Chapter 8. An Investigation into Serum Blood Lipids and Radiographic OA, Musculoskeletal Pain and Chronic Widespread Pain.	TWINS-UK	Cross-sectional	Cohort Study	Multi-level Mixed Effects Logistic Regression
Chapter 9. An Investigation into Dietary and Lifestyle Factors and Change and Incidence of Hip and Knee Pain.	EPIC-Norfolk	Longitudinal	Case-control Study	Regression analysis with Stepwise Algorithm and Randomisation Function.
Chapter 10. Investigating Cause and Effect. An Analysis of Single Nucleotide Polymorphism Data from the EPIC-Norfolk Cohort.	EPIC-Norfolk	Cross sectional	Mendelian Randomisation Study	Two-stage least squares regression

2.3 Aims and Objectives

This project aims to contribute to the existing literature and give further valuable insight into the effects of diet on OA and pain by analysing data within the two cohorts described previously, EPIC-Norfolk and TWINS-UK.

This thesis aims to:

1. Further our understanding of the relationship between lifestyle and dietary factors and osteoarthritis of weight bearing joints such as the hip and knee and of non-weight bearing joints such as the hand.
2. Further our understanding of the relationship between lifestyle and dietary factors and joint pain associated with OA, again in both weight bearing joints (such as the knee, hip and foot) and non-weight bearing joints (such as the hand, elbow and shoulder).
3. Further understand the direction of causality between explanatory factors of interest and OA/pain.

The objectives of this research are to:

1. Investigate the relationship between lifestyle and dietary factors and clinical OA of the hip and knee in the EPIC-Norfolk cohort.
2. Using joint pain as a proxy for OA, investigate associations between lifestyle and dietary factors and joint pain of the hip and knee in EPIC-Norfolk.
3. Further investigate the relationship between diet and radiographic OA of the hip, knee and hand by analysing PCA derived dietary patterns created from FFQ data in TWINS-UK.
4. Using joint pain as a proxy for OA and chronic widespread pain as a proxy for joint pain, investigate associations between diet and musculoskeletal pain variables in TWINS-UK.
5. Investigate further any initial exploratory cross-sectional associations found in EPIC using available data from TWINS-UK.
6. Using EPIC-Norfolk Longitudinal joint pain data, further investigate any dietary associations found from the cross-sectional analysis of OA/pain in order to replicate and validate findings.
7. Request genetic data from EPIC-Norfolk and use a Mendelian Randomisation approach to better understand in which direction relationships may occur to produce findings on selected SNPs of interest and their association with OA.

Figure 9 shows the flow of the following chapters, starting with cross-sectional analysis investigating EPIC variables to explore those factors that best predict OA and musculoskeletal pain. Due to female gender influencing the onset and progression of OA/pain, further analysis took place to investigate these same factors in separate analysis for males and females and with females stratified by age. TWINS-UK provides extensive dietary data so analysis took place to investigate associations between dietary patterns and OA/musculoskeletal pain and the dietary data provided the chance to create a Mediterranean dietary pattern to investigate OA and pain. Due to the results from the EPIC and TWINS-UK analysis the effects of blood lipids were also investigated. However whether an association is in fact casual is difficult to determine throughout cross-sectional analysis so further analysis of a longitudinal design took place in EPIC with the final chapter using single nucleotide polymorphisms to investigate reverse causality.

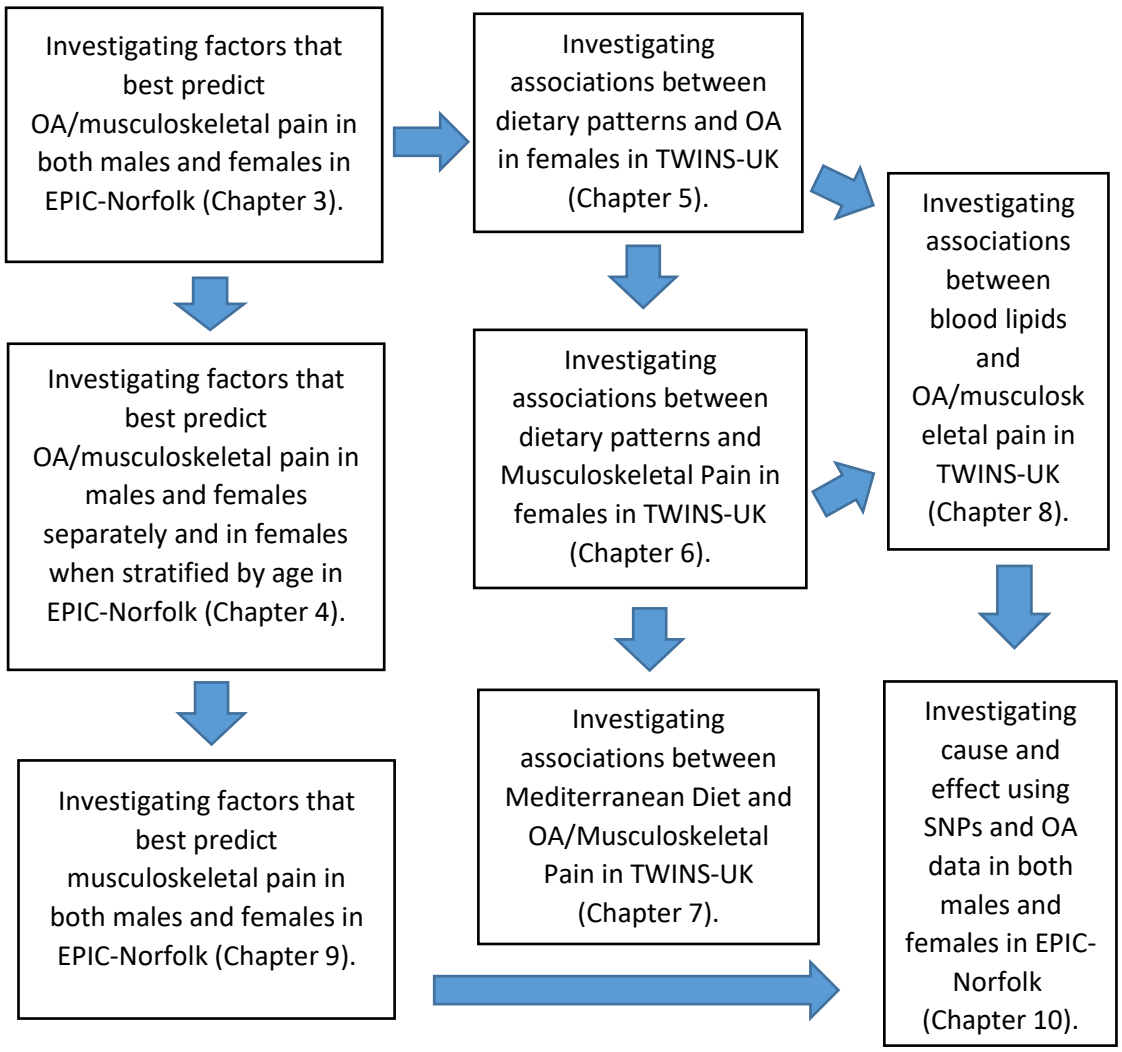

FIGURE 9. FLOWCHART SHOWING HOW THE CHAPTERS CONTAINING ANALYSIS IN THIS THESIS ARE LINKED.

3 An Investigation into associations between Dietary and Lifestyle Factors and Osteoarthritis/Pain of the Hip and Knee. A Case-Control Analysis using Data from EPIC-Norfolk.

3.1 Chapter Overview

OA and musculoskeletal pain are subject to influence from multiple lifestyle and dietary factors as discussed in Chapter 1. Previous research has suggested that both dietary manipulation and modification of lifestyle factors are ways in which patients can help themselves to alleviate symptoms. This chapter investigates associations within the EPIC-Norfolk cohort, using a case-control analysis of cross-sectional design to explore those factors that best predict clinical OA and pain of the hip and knee. The main findings from this analysis is that age and BMI are consistently associated with OA and pain. Consumption of fatty foods is also positively associated with hip and knee OA and Triglycerides are positively associated with knee OA and knee pain.

3.2 Introduction

EPIC-Norfolk is one of two UK centres within EPIC. The EPIC-Norfolk cohort contains dietary and lifestyle data alongside clinical OA and pain data of the hip and knee. This allows the opportunity to investigate the aims of this project using a large cohort, containing both male and female participants.

EPIC-Norfolk included a baseline health examination (1st health check) of 25,639 men and women, aged 40–79 years of age. These volunteers were recruited between 1993 and 1997, from East Anglia, via general practice registers. At baseline, there exists nutritional data and clinical OA data for diagnosis of hip and knee OA. Participants were invited to a follow-up assessment (2nd health check), between 1998 and 2000, which included the same tests as undertaken at baseline in addition to further variables. Between 2006 and 2011, the 3rd health check occurred with 8623 participants (aged 48–92 years), to investigate conditions relevant to ageing (Day, Oakes et al. 1999) (see Figure 10). Lifestyle and pain data were therefore collected at three different time points post-baseline but there is no pain data available at baseline so this cross-sectional analysis uses the pain data collected at the first health check (the first time point at which pain data became available).

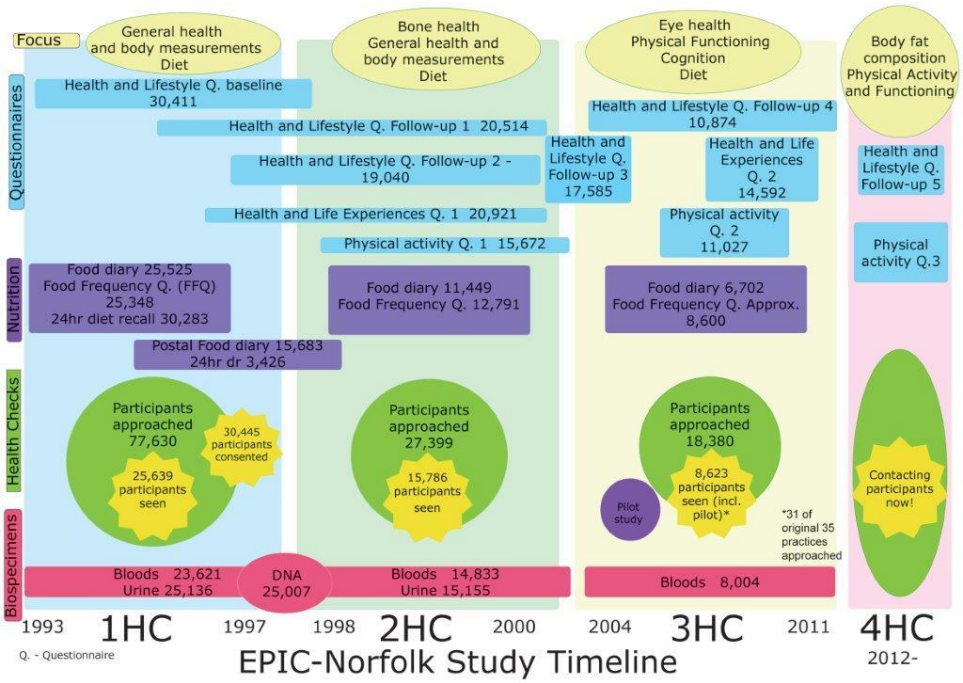

FIGURE 10. EPIC-NORFOLK TIMELINE OF DATA COLLECTION (WWW.MRC-EPID.CAM.AC.UK/RESEARCH/STUDIES/EPIC-NORFOLK).

Figure 10 outlines when the EPIC-Norfolk data, for each of the variables of interest, was collected and the method by which this was done.

Data collected from FFQs and lifestyle questionnaires was requested from EPIC-Norfolk to investigate the effects of lifestyle and dietary factors on OA and pain. This aimed to determine, to what extent, diet plays a role in predicting the onset of disease and also explore those lifestyle factors that best predict the onset of OA/pain. This subset of the EPIC-Norfolk cohort differs from TWINS-UK (investigated in the following chapters) in that it has a larger number of participants, contains both male and female participants and contains data on hip pain but not hand pain. EPIC-Norfolk also contains pain data at multiple time points, enabling a longitudinal approach (please see Chapter 9).

The EPIC-Norfolk cohort, therefore, provides a good opportunity to conduct an exploratory analysis to investigate which dietary factors alongside lifestyle factors best predict clinical OA and musculoskeletal pain.

3.3 Methods

3.3.1 Clinical OA Data

Hip OA and Knee OA variables were gained by linking existing EPIC-Norfolk data from participants to the Hospital Episode Statistics (HES) Database which holds information on 20, 517 patients, some of whom have been diagnosed with OA and some of whom have undergone surgery for end stage OA. The HES OA data for EPIC-Norfolk analysis was coded 0 for no diagnosed OA and 1 for any degree of OA severity. Table 2 below shows the numbers for the presence and absence of knee and hip OA within the EPIC-Norfolk cohort.

TABLE 2. CHARACTERISTICS OF EPIC-NORFOLK HES OA DATA.

OA variable	Present	Absent
Knee OA	789	19728
Hip OA	680	19837

3.3.2 Pain Data

Pain data within EPIC-Norfolk was collected at multiple time points: 18 months post baseline (first health check), 3 years post baseline (second health check) and 10 years post baseline (third health check). The pain data used for the cross-sectional analysis (Table 3 below) was that of the first health check post baseline.

TABLE 3. CHARACTERISTICS OF PAIN DATA AT FIRST HEALTH CHECK.

	Pain	No Pain	Missing
Hip Pain	2467	17199	850
Knee Pain	3886	15897	733

Knee, hip and back pain in EPIC-Norfolk is defined as 'Have you had hip/knee pain on most days of the last month?'

3.3.3 Dietary Data

Using previously developed and validated methods (Willett 2012), dietary intake was assessed in EPIC-Norfolk. The method which represents the dietary data used in this study was collected via a semi-quantitative food-frequency questionnaire. A 130-item, semi-quantitative FFQ, based on the FFQ used in the Nurses' Health Study, was designed and used at the 1st health check to evaluate the habitual diet of participants over the past year.

Participants were asked how often they consumed each food, on average, within the last year of which the options were:

- Never or less than once per month
- 1-3 times per month
- Once a week
- 2-4 times per week
- 5-6 times per week
- Once a day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Further questions on type and brand of foods and method of cooking followed and participants were instructed not to leave any questions within the FFQ blank but to estimate as best they could. Food intake values were then calculated from the FFQ data through the use of Compositional Analyses from Frequency Estimates (CAFE) program and extreme outliers of nutrient intake were flagged when identifying the top and bottom 0.5% of the ratio of energy intake to estimated basal metabolic rate.

The range of EPIC FFQ data available to use for this thesis was limited and dietary intakes were recorded as a combination of both weight of food (g/day) and portions of food (per day/week) where food groups were classified as the following: 1= Never, 2= Seldom, 3= Once a week, 4= 2-4 times a week, 5= 5-6 times a week, 6= once or more daily (see tables below).

To gain blood lipid data, non-fasting blood samples were taken by venepuncture. Serum concentrations of total cholesterol and HDL were measured with RA 1000 Technicon analyser and when triglycerides did not exceed 4mmol/l LDL was calculated using the Friedewald formula.

3.3.4 Characteristics of EPIC-Norfolk Data

TABLE 4. CHARACTERISTICS OF CONFOUNDERS IN EPIC-NORFOLK DATA.

		Missing data
Total	20517	
Gender		3
Females	11594	
Males	8920	
Mean Age (years)	60	7
Mean BMI (kg/m²)	26.2	2204
Physical Activity		3
Active	8243	
Inactive	12269	
Alcohol Consumption		640
Use	18582	
Never use	1295	
Smoking		179
Current-use	2074	
Former-use	8498	
Never-use	9765	
HRT		8931
Current-use	2223	
Former-use	1268	
Never-use	8031	

The EPIC-Norfolk cohort is useful for Investigating Osteoarthritis/Pain as the mean age of 60 (as above) is when onset and progression is typically seen and, as can be seen from Tables 5-8, the mean age of those with OA/Pain cases is higher than the average for non-cases for all outcome variables. With mean BMI at 26 (as in Table 4 above) this cohort is also representative of an overweight population which, again, is useful for the investigation of Osteoarthritis and associated pain as onset and progression are seen with increasing BMI. As can be seen from Tables 5-8 below the average BMI for OA/Pain cases is higher than non-cases. Within the EPIC-Norfolk cohort there are also more females than males and 18582 participants (out of a total 20517) reported consuming alcohol on a regular basis. The number who reported smoking currently or being a former smoker was also higher than those who had never smoked in both cases and non-cases and numbers of those who reported being active or moderately active were lower in both cases and non-cases than those who were inactive. The average levels of blood lipids were also elevated for all outcome variables with the exception of HDL which was shown to be lower in knee OA and knee pain cases than non-cases (see Tables 5-8).

TABLE 5. CHARACTERISTICS OF EPIC-NORFOLK DATA BY OA SITE.

Knee OA	**OA Cases**				**Non cases**			
	mean	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	64	41	79	8	60	40	80	9
BMI (kg/m²)	28.7	19.6	52.7	4.5	26.1	15.5	49.7	3.8
Energy Intake (kj)	8578.03	3632.77	17726.41	2422.74	8616.52	3075.29	21409.25	2462.87
Cholesterol (mmol/l)	6.37	2.70	12.00	1.24	6.19	2.10	18.00	1.17
Triglycerides (mmol/l)	1.99	0.50	10.60	1.15	1.79	0.20	26.00	1.10
HDL (mmol/l)	1.40	0.50	5.90	0.43	1.43	0.20	4.40	0.42
LDL (mmol/l)	4.10	0.99	9.52	1.09	3.97	0.45	10.30	1.04
Energy Adjusted Dietary Data								
Allium Vegetables	0.003	0.000	0.022	0.003	0.003	0.000	0.031	0.002
Cruciferous Vegetables	0.011	0.000	0.074	0.008	0.010	0.000	0.143	0.007
Green Vegetables	0.002	0.000	0.016	0.002	0.002	0.000	0.043	0.002
Yellow Vegetables	0.007	0.000	0.054	0.004	0.007	0.000	0.078	0.004
Other Vegetables	0.005	0.000	0.028	0.004	0.005	0.000	0.052	0.004
Citrus Fruits	0.007	0.000	0.077	0.008	0.006	0.000	0.125	0.008
Non Citrus Fruits	0.024	0.000	0.135	0.018	0.023	0.000	0.176	0.017
Chips	0.004	0.000	0.022	0.003	0.004	0.000	0.039	0.003
Dietary Data (portions/week)								
Fruits	5.11	1	6	1.18	4.97	1	6	1.27
Leafy Vegetables	4.60	1	6	1.03	4.46	1	6	1.05
Fatty Fish	2.57	1	6	0.92	2.46	1	6	0.85

Other Fish	3.06	1	6	0.70	3.02	1	6	0.70
Chicken	3.33	1	6	0.75	3.27	1	6	0.79
Meat	3.58	1	6	0.87	3.47	1	6	0.95
Meat Products	2.80	1	6	0.88	2.83	1	6	0.90
Eggs	3.23	1	6	0.88	3.21	1	6	0.91
Cheese	3.77	1	6	1.11	3.84	1	6	1.10
Brown Bread	4.33	1	6	1.77	4.32	1	6	1.76
Milk (1(None), 2(<=1/2 pints/week), 3(>1/2 pints/week & <1/2pint/day), 4(1/2 to 1 pint/day), 5(more than 1 pint daily).	3.65	1	5	0.89	3.67	1	5	0.86
Snacks (No. of times a day you eat including meals, snacks, coffee breaks etc.)	3.90	0	8	1.10	3.97	0	15	1.20
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	298	490			7947	11778		
	Current	*Never*			*Current*	*Never*		
Alcohol	713	58			17869	1237		
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	61	354	362		2013	8144	9403	
HRT	78	69	326		2145	1199	7765	
Hip OA								
	mean	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	64	40	78	8	60	40	80	9
BMI (kg/m²)	27.7	18.1	52.7	4.6	26.1	15.5	49.7	3.8
Energy Intake (kj)	8410.40	3807.97	16482.69	2325.43	8622.01	3075.29	21409.25	2465.55

Cholesterol (mmol/l)	6.34	3.60	10.20	1.13	6.19	2.10	18.00	1.18
Triglycerides (mmol/l)	1.86	0.40	9.30	1.04	1.80	0.20	26.00	1.10
HDL (mmol/l)	1.46	0.50	3.00	0.44	1.43	0.20	5.90	0.42
LDL (mmol/l)	4.05	1.83	7.15	1.00	3.97	0.45	10.30	1.04
Energy Adjusted Dietary Data								
Allium Vegetables	0.003	0.000	0.022	0.003	0.003	0.000	0.031	0.002
Cruciferous Vegetables	0.012	0.000	0.143	0.010	0.010	0.000	0.108	0.007
Green Vegetables	0.002	0.000	0.043	0.003	0.002	0.000	0.035	0.002
Yellow Vegetables	0.007	0.000	0.078	0.006	0.007	0.000	0.069	0.004
Other Vegetables	0.005	0.000	0.037	0.004	0.005	0.000	0.052	0.004
Citrus Fruits	0.006	0.000	0.062	0.008	0.006	0.000	0.125	0.008
Non Citrus Fruits	0.026	0.000	0.157	0.019	0.023	0.000	0.176	0.017
Chips	0.003	0.000	0.026	0.003	0.004	0.000	0.039	0.003
Dietary Data (portions/week)								
Fruits	5.13	1	6	1.21	4.97	1	6	1.27
Leafy Vegetables	4.63	1	6	1.01	4.45	1	6	1.05
Fatty Fish	2.58	1	6	0.91	2.46	1	6	0.85
Other Fish	3.10	1	6	0.73	3.02	1	6	0.70
Chicken	3.33	1	6	0.81	3.27	1	6	0.78
Meat	3.55	1	6	0.92	3.47	1	6	0.95
Meat Products	2.81	1	6	0.90	2.83	1	6	0.90
Eggs	3.25	1	6	0.88	3.21	1	6	0.91
Cheese	3.78	1	6	1.08	3.83	1	6	1.10
Brown Bread	4.37	1	6	1.77	4.31	1	6	1.76

Milk (1(None), 2(<=1/2 pints/week), 3(>1/2 pints/week & <1/2pint/day), 4(1/2 to 1 pint/day), 5(more than 1 pint daily).	3.70	1	5	0.89	3.66	1	5	0.86
Snacks (No. of times a day you eat including meals, snacks, coffee breaks etc.)	3.88	0	9	1.18	3.98	0	15	1.26
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	240	439			8004	11830		
	Current	*Never*			*Current*	*Never*		
Alcohol	602	54			17980	1241		
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	47	324	301		2027	8175	9462	
HRT	64	45	320		2159	1223	7770	

TABLE 6. CHARACTERISTICS OF EPIC-NORFOLK DATA BY PAIN SITE.

Knee Pain	**Pain Cases**				**Non cases**			
	mean	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	61	40	79	9	59	40	80	9
BMI (kg/m²)	27.3	15.5	50.0	4.3	25.8	16.1	49.6	3.6
Energy Intake (kj)	8678.40	3119.89	19812.46	2567.17	8619.72	3075.29	21409.25	2439.71
Cholesterol (mmol/l)	6.26	2.10	11.40	1.18	6.17	2.60	18.00	1.17
Triglycerides (mmol/l)	1.89	0.30	13.10	1.08	1.77	0.20	26.00	1.10
HDL (mmol/l)	1.41	0.60	4.00	0.42	1.43	0.20	4.40	0.42
LDL (mmol/l)	4.01	0.91	8.83	1.04	3.96	0.45	10.30	1.04

Energy Adjusted Dietary Data								
Allium Vegetables	0.002	0.000	0.025	0.002	0.003	0.000	0.031	0.002
Cruciferous Vegetables	0.011	0.000	0.108	0.008	0.010	0.000	0.074	0.007
Green Vegetables	0.002	0.000	0.035	0.002	0.002	0.000	0.035	0.002
Yellow Vegetables	0.007	0.000	0.063	0.004	0.007	0.000	0.069	0.004
Other Vegetables	0.005	0.000	0.045	0.004	0.005	0.000	0.052	0.004
Citrus Fruits	0.006	0.000	0.125	0.008	0.006	0.000	0.096	0.008
Non Citrus Fruits	0.023	0.000	0.141	0.018	0.023	0.000	0.176	0.017
Chips	0.004	0.000	0.024	0.003	0.004	0.000	0.035	0.003
Dietary Data (portions/week)								
Fruits	4.90	1	6	1.31	4.99	1	6	1.26
Leafy Vegetables	4.46	1	6	1.07	4.45	1	6	1.05
Fatty Fish	2.50	1	6	0.87	2.45	1	6	0.84
Other Fish	3.03	1	6	0.73	3.01	1	6	0.69
Chicken	3.28	1	6	0.79	3.26	1	6	0.78
Meat	3.47	1	6	0.94	3.46	1	6	0.96
Meat Products	2.83	1	6	0.90	2.83	1	6	0.90
Eggs	3.23	1	6	0.92	3.21	1	6	0.91
Cheese	3.82	1	6	1.08	3.84	1	6	1.10
Brown Bread	4.25	1	6	1.79	4.33	1	6	1.75
Milk (1(None), 2(<=1/2 pints/week), 3(>1/2 pints/week & <1/2pint/day), 4(1/2 to 1 pint/day), 5(more than 1 pint daily).	3.67	1	5	0.84	3.67	1	5	0.84
Snacks (No. of times a day you eat including meals, snacks, coffee breaks etc.)	1.03	0	3	0.96	1.03	0	3	0.96
	Active	*Inactive*			*Active*	*Inactive*		

Physical Activity	1200	2008			6328	8931		
	Current	*Never*			*Current*	*Never*		
Alcohol	2877	235			13891	898		
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	372	1418	1387		1519	6174	7450	
HRT	385	263	1247		1618	859	5924	
Hip Pain								
	mean	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	61	41	78	9	59	40	80	9
BMI (kg/m²)	27.0	16.1	49.1	4.1	25.9	15.5	50.0	3.7
Energy Intake (kj)	8504.59	3119.89	19481.25	2524.93	8650.55	3075.29	21409.25	2454.52
Cholesterol (mmol/l)	6.34	2.60	11.40	1.20	6.16	2.10	18.00	1.17
Triglycerides (mmol/l)	1.90	0.30	13.10	1.18	1.77	0.20	26.00	1.09
HDL (mmol/l)	1.44	0.60	4.00	0.42	1.42	0.20	4.40	0.42
LDL (mmol/l)	4.07	1.10	8.91	1.07	3.95	0.50	10.30	1.04
Dietary Data (g/day)								
Allium Vegetables	0.003	0.000	0.025	0.003	0.002	0.000	0.031	0.002
Cruciferous Vegetables	0.011	0.000	0.108	0.008	0.010	0.000	0.081	0.007
Green Vegetables	0.002	0.000	0.022	0.002	0.002	0.000	0.035	0.002
Yellow Vegetables	0.007	0.000	0.057	0.005	0.006	0.000	0.069	0.004
Other Vegetables	0.005	0.000	0.040	0.004	0.005	0.000	0.052	0.004
Citrus Fruits	0.006	0.000	0.061	0.008	0.006	0.000	0.125	0.008
Non Citrus Fruits	0.024	0.000	0.157	0.018	0.023	0.000	0.176	0.017

Chips	0.004	0.000	0.039	0.003	0.004	0.000	0.035	0.003
Dietary Data (portions/week)								
Fruits	4.92	1	6	1.31	4.98	1	6	1.26
Leafy Vegetables	4.49	1	6	1.10	4.45	1	6	1.05
Fatty Fish	2.48	1	6	0.87	2.46	1	6	0.84
Other Fish	3.03	1	6	0.70	3.02	1	6	0.70
Chicken	3.29	1	6	0.79	3.27	1	6	0.78
Meat	3.45	1	6	0.98	3.46	1	6	0.96
Meat Products	2.79	1	6	0.89	2.84	1	6	0.90
Eggs	3.20	1	6	0.93	3.22	1	6	0.91
Cheese	3.76	1	6	1.11	3.85	1	6	1.09
Brown Bread	4.24	1	6	1.80	4.33	1	6	1.75
Milk (1(None), 2(<=1/2 pints/week), 3(>1/2 pints/week & <1/2pint/day), 4(1/2 to 1 pint/day), 5(more than 1 pint daily).	3.67	1	6	0.96	3.70	1	6	0.87
Snacks (No. of times a day you eat including meals, snacks, coffee breaks etc.)	1.03	0	3	0.98	1.03	0	3	0.96
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	711	1334			6791	9531		
	Current	*Never*			*Current*	*Never*		
Alcohol	1819	171			14858	958		
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	249	898	880		1635	6649	7910	
HRT	315	204	850		1687	920	6275	

3.3.5 Energy Intake

It is important in nutritional epidemiology to adjust for total energy intake. This factor, also known as caloric intake, needed to be adjusted for as it is used to take into account the individual differences in energy intake (of which the many associated variables can be body size, metabolic efficiency and net energy balance) which is extremely important in all nutritional epidemiological research (Willett and Stampfer 1986). The EPIC-Norfolk FFQ dietary variables were, therefore, adjusted for total energy intake by dividing the FFQ nutritional variable (mass per day) by the FFQ energy intake (kj/day) to create a new adjusted variable for each FFQ dietary component.

3.3.6 Statistical Analysis

Using a case-control design, the independent variables, outlined below, were investigated against the dependent variables; hip and knee OA and hip and knee pain. After defining the group for each of these outcome variables, random selection of participants for the control group took place to ensure a fair predictive value using a simple randomisation function in R. This selected the appropriate number of controls to cases (1:1 ratio) by separating all potential controls into one group and then randomly selecting the number required using the 'sample' function in the base package of R. This function uses a random number generator (RNG) to select data from a list providing data for a case-control analysis.

Nutritional and lifestyle variables were selected due to them having been found to influence OA and pain, as discussed in Chapter 1. These were modelled using a logistic regression analysis with a stepwise algorithm, as discussed in Chapter 2. This analysis investigates the relationship between the dependent variables (clinical hip and knee OA and joint pain of the hip and knee) and independent variables of interest. Lifestyle variables include age, BMI, gender, waist circumference, hip circumference, height, smoking status, teetotal, alcohol intake, ethnic group, physical activity, cholesterol, triglycerides, HDL and LDL. Dietary variables of interest include citrus fruits, non-citrus fruits, leafy vegetables, other vegetables, allium vegetables, cruciferous vegetables, green vegetables, yellow vegetables, fatty fish, other fish, chicken, meat, meat products, eggs, cheese, brown bread, milk, snacks, chips and energy intake. The analysis was then repeated but using only the nutrition related variables.

Due to the limited availability of dietary variables in this subset of EPIC-Norfolk data, dietary patterns were not investigated.

3.4 EPIC-Norfolk Predictors of OA and Musculoskeletal Pain Results

3.4.1 Predictors of Hip OA

This analysis randomly selected those who did not have hip OA (n=666) as control against those who have hip OA (n=680) (see methods). Table 7 shows the results for the significant explanatory factors of hip OA.

TABLE 7. SIGNIFICANT EXPLANATORY VARIABLES FOR HIP OA IN EPIC-NORFOLK.

Significant predictors	OR	95% CI
Age	1.07	(1.05, 1.09)
BMI	1.18	(1.13, 1.24)
Chips	0.80	(0.72, 0.89)

Analysis of independent variables of interest showed that increased age leads to an increased risk of hip OA ($p<0.0001$). BMI shows that an increased BMI leads to an increased risk of hip OA ($p<0.0001$). Consumption of chips is also significantly negatively associated with hip OA ($p<0.0001$).

3.4.2 Predictors of Knee OA

Consistent with the analysis for hip OA (Table 7), random selection of participants who did not have knee OA (n=765) were selected as controls against those who have knee OA (n=789) (see methods). Table 8 (below) shows the results of those risk factors found to be significantly associated with knee OA.

TABLE 8. SIGNIFICANT EXPLANATORY VARIABLES FOR KNEE OA IN EPIC-NORFOLK.

Significant predictors	OR	95% CI
Age	1.06	(1.05, 1.08)
BMI	1.21	(1.17, 1.25)
Leafy Vegetables	1.27	(1.11, 1.46)
Other vegetables	0.83	(0.71, 0.97)
Fatty Fish	1.20	(1.05, 1.38)
Triglycerides	1.13	(1.02, 1.25)

Age is a significant predictor of knee OA and shows that increased age leads to an increased risk of knee OA ($p<0.0001$). BMI is a significant predictor of knee OA and shows that increased BMI leads to an increased risk of knee OA ($p<0.0001$)).

Consumption of leafy Vegetables is significantly positively associated with knee OA ($p<0.001$). The food group 'other vegetables' is significantly negatively associated with knee OA. ($p=0.023$). Fatty Fish is significantly positively associated with knee OA ($p=0.008$) and triglycerides is also significantly positively associated with knee OA ($p=0.023$).

3.4.3 Predictors of Hip Pain

There are approximately 2000 cases of hip pain so 2000 non-cases were randomly selected to form the combined dataset (see methods). Table 9 shows the results of those factors found to be most significantly associated with hip pain.

TABLE 9. SIGNIFICANT EXPLANATORY VARIABLES FOR HIP PAIN IN EPIC-NORFOLK.

Significant explanatory variables	OR	95% CI
Age	1.03	(1.02, 1.03)
BMI	1.06	(1.04, 1.08)
Gender	1.95	(1.71, 2.22)
Smoking status	0.58	(0.46, 0.72)
Teetotal	0.79	(0.67, 0.94)).
Energy intake	1.00	(1.00, 1.00)
Cruciferous vegetables	1.11	(1.05, 1.18)

These findings suggest that increased age increases the risk of hip pain ($p<0.0001$). Having female gender also increases the risk of hip pain ($p<0.0001$). Being a non-smoker is associated with hip pain and suggests a decrease in the risk of hip pain ($p<0.001$). Not being teetotal is also significantly associated with hip pain and suggests a decrease in the risk of hip pain ($p=0.01$). Increased BMI was found to increase the risk of hip pain ($p<0.001$). Repeating the analysis using only the nutrition related variables, increased intake of calories was found to be positively associated with the risk of hip pain ($p=0.040$). Increased intake of cruciferous vegetables was also found to be positively associated with the risk of hip pain ($p<0.001$).

3.4.4 Predictors of Knee Pain

There are about 3000 cases of knee pain so 3000 non-cases were randomly selected to form the combined dataset (see methods). Table 10 shows those variables that are significant predictors of knee pain.

TABLE 10. SIGNIFICANT EXPLANATORY VARIABLES FOR KNEE PAIN IN EPIC-NORFOLK.

Significant explanatory variables	OR	95% CI
Age	1.30	(1.02, 1.03)
BMI	1.11	(1.10, 1.13)
Gender	1.30	(1.17, 1.44)
Smoking status	0.62	(0.52, 0.74)
Triglycerides	1.15	(1.10, 1.21)

These findings suggest that increased age increases the risk of knee pain ($p<0.0001$). Having female gender increases the risk of knee pain ($p<0.0001$). Being a non-smoker is significantly negatively associated with the risk of knee pain ($p<0.0001$). Increased BMI also suggests an increase in the risk of knee pain ($p<0.0001$). Repeating the analysis using only the nutrition related variables, the results showed that higher triglycerides is positively associated with the risk of knee pain ($p<0.0001$).

3.5 Discussion

The result that age and BMI are significantly associated with hip OA is the result of a statistical model which, based on the pseudo r^2 value, explains approximately 9% of the variation in the data. This result supports previous findings that show that obesity is

positively associated with hip OA (Lievense, Bierma-Zeinstra et al. 2002). The results from the case-control analysis of knee OA showed that increased age and BMI are significantly associated with knee OA and this model explains approximately 13% of the variation in the data. This finding also supports those found in previous studies. BMI is considered to be an important contributing factor to the development of knee OA (Blumenfeld, Williams et al. 2013) and BMI has been found to be the strongest risk factor for incidence and progression of knee OA (Jonsson, Helgadottir et al. 2011).

Alongside age, gender, smoking status and being teetotal, BMI was also found to be significantly associated with hip pain with all five of these predictors of hip pain explaining approximately 5% of the variation. The result that age, gender, smoking status and BMI are associated with knee pain also explains approximately 5% of the variation in knee pain.

The results from the dietary analysis for knee OA highlighted some key results, however, this explains less than 2% of the variation and further to this less than 0.5% of the variation in knee pain is explained by the result that increased triglycerides and increased consumption of cruciferous vegetables is positively associated with knee pain.

An increase in triglycerides was found to be positively associated with risk of knee OA ($p=0.023$) (Table 8) and risk of knee pain ($p<0.0001$) (Table 10). Triglycerides are lipid components of chylomicrons which are synthesised and secreted by intestinal enterocytes. They are also found within very low density lipoprotein which is synthesised and secreted by the liver (Khetarpal and Rader 2015). Lipoprotein lipase (LPL), produced by multiple tissues including adipose tissue, is required for the hydrolysis of these triglyceride rich lipoproteins. The reaction products, monoacylglycerol and fatty acids are then taken up and stored by adipose tissue, muscle and macrophages. It is important to consider the actions of LPL (due to diet and hormonal status being able to affect LPL and its associated proteins) as the function of this enzyme regulates the supply of fatty acids to its target tissues (Wang and Eckel 2009).

The finding that not being teetotal is suggested to decrease the risk of hip pain (Table 9) is an interesting result and there have been many studies investigating the effects of alcohol consumption on health. There exists much speculation as to the reasons why excessive alcohol consumption increases risk of disease but moderate consumption has been found to have beneficial effects compared to no alcohol consumption which results in a J shaped curve (Plunk, Syed‐Mohammed et al. 2014). The finding that not being teetotal decreases risk of pain can therefore be explained by the idea that complete abstinence from alcohol consumption is not as beneficial as a small amount of exposure to the substance.

A study that investigated lipid accumulation in patients with hypertension, found that both U and J shaped relationships existed between intake of alcohol and lipid accumulation products, suggesting that a light to moderate amount of exposure is more beneficial than no exposure (Wakabayashi 2015). A moderate consumption of alcohol has been found to inhibit lipopolysaccharide activation of NF-κB regulating expression of *TNF-α* and *IL-1β (Mandrekar, Catalano et al. 2006).* The cytokine IL-10 (which has been found to have anti-inflammatory effects due to its ability to inhibit TNF-α (Wang, Wu et al. 1994)), was also heightened by moderate intake of alcohol (Mandrekar, Catalano et al. 2006). This suggests that moderate amounts of alcohol could be protective of inflammation and there are, therefore, multiple mechanisms by which the association found in this research could be explained.

The cross-sectional analysis in this chapter also found that smoking status is associated with hip pain (Table 9). This finding suggests that being a non-smoker decreases the risk of hip pain ($p<0.001$). CRP levels are elevated in OA and could be related to OA symptoms, such as loss of function and onset of pain, in those suffering from OA (Jin, Beguerie et al. 2015). Cigarette smoking has also been found to be positively associated with serum high sensitivity CRP levels in men and women suffering from the early stages of radiographic knee OA (Zhang, Zeng et al. 2016).

Cigarette smoke is known to interfere with mechanisms involved in the inflammatory response, for instance the SIRT1 and Rel protein interaction which causes activation of NF-κB in macrophages (Yang, Wright et al. 2007). However, there is conflicting evidence as to the effect smoking has on OA. Smokers have been found to have more severe and persistent musculoskeletal pain than non-smokers (Felson and Zhang 2015) as nicotine has pro-nociceptive effects through the CNS. The dorsal route ganglion contains many nicotine sensitive acetylcholine receptors and increased pain sensitivity occurs from downregulation of the hypothalamic pituitary axis, all as a result of smoking (Miao, Green et al. 2004).

The 1958 British Birth Cohort Study found that both past and current smoking history (as well as elevated BMI) and a more physically demanding job, were all associated with CWP in both men and women. Addiction to tobacco is a risk factor for chronic pain, however, those who live with conditions where the main symptom is pain are more likely to become dependent on nicotine questioning the direction of causality (Bakhshaie, Ditre et al. 2016). Some studies have found that being a current smoker is associated with OA (Haugen, Magnusson et al. 2017) but other studies have found inverse associations between smoking and OA. A mechanism behind this is that smoking prevents weight gain (Felson and Zhang 2015) and may, therefore, have a protective effect on osteoarthritis. However many of the studies that show such associations are unlikely to represent true associations due to bias (Hui, Doherty et al. 2011). Even if a true association existed, smoking tobacco products would not be recommended due to the number of negative health effects but, similarly to looking at diet, research is ongoing to understand the effects of components, if indeed there does exist a protective effect (Felson and Zhang 2015).

Fried foods are found in high volumes in a westernised world of fast food culture and a substantial part of the diet is consumption of chips. The process of frying foods causes them to become crunchy and aromatic improving palatability which can lead to over consumption (Drewnowski and Almiron-Roig 2009).

The process of frying foods also modifies fatty acid composition. The oils used deteriorate and the degradation products are absorbed into the fried food, leading to the ingestion of higher amounts of oxidised fatty acids (Casal, Malheiro et al. 2010). This is due to the process of oxidation and hydrogenation, causing a loss of unsaturated fats and an increase in trans-fats (Fillion and Henry 1998). This causes the fried food to increase in energy density whilst reducing in water content (Guallar-Castillón, Rodríguez-Artalejo et al. 2012).

The health risks of consuming trans-fats are due to the changes they induce and incorporation into the phospholipid membranes of cells, altering transport and cellular signalling. In addition COX and lipoxygenase enzymes cannot metabolise trans-fatty acids as they do not recognise the unusual shape (Ginter and Simko 2016).

The observed association with chips is unexpected due to the negative effects of trans- fats on cellular and inflammatory processes, however, the formation of resistant starch

(through frying potato) also alters the dietary fibre content of this food (Fillion and Henry 1998).

Dietary fibre intake has been found to be closely related to body composition/BMI and inflammation. A recent study found inverse associations between dietary fibre and body composition in multiple food sources, with the exception of potato fibre intake (Gibson, Eriksen et al. 2019). The changes that take place in starch-rich foods through heating and cooling are known as retrogradation (Fredriksson, Björck et al. 2000). For example, retrograded amylase and starch known as resistant starch III (RSIII) can form when starchy foods are processed, but more studies are needed to investigate the effects of this digestion resistant carbohydrate (Gibson, Eriksen et al. 2019).

Fried foods and their effects on health have been investigated widely. A longitudinal study (over an eight year period) investigating the effects of fried potato and their effect on mortality found that consumption of fried potato, twice a week or more, was associated with an increased risk of mortality. In contrast, the consumption of potato that had not been fried was not associated with increased mortality (Veronese, Stubbs et al. 2017). The inverse association of consumption of chips with hip OA observed could, therefore, be explained by the fact that diet may have been altered in those suffering from hip OA.

Analysis of EPIC-Norfolk dietary variables showed a positive association between consumption of fatty fish and knee OA. Deep fried fish related foods can be separated into those with a battered coating and those without (He, Franco et al. 2015). The fat uptake of deep fried raw fish increases from 1.4% to 18%, however, this is not as much of an increase as that seen in other fried foods, such as potato chips (Makinson, Greenfield et al. 1987). Studies completed over the last few years have been investigating how changing the ingredients in batter (to those with a low oil binding capacity) could reduce the uptake of fat (He, Franco et al. 2015).

Oils have also been investigated for their frying abilities and shelf life by looking specifically at their stability under frying conditions. Olive oil (the oil of choice within the Mediterranean dietary pattern) has been found to be highly resistant to oxidation which could be due in part to the phenolic compounds found within it (Casal, Malheiro et al. 2010). An EPIC study that investigated fried food consumption when the oils used were those within a Mediterranean diet (such as olive oil), found that consumption of fried foods was not found to be associated with coronary heart disease or mortality (Guallar-Castillón, Rodríguez-Artalejo et al. 2012). However, other studies have found fried food consumption to be associated with a higher risk of hypertension despite investigating effects within a Mediterranean cohort (Sayon-Orea, Bes-Rastrollo et al. 2014). An increased or more frequent consumption of fried foods has been found to be significantly associated with increased risk of type-2 diabetes (Cahill, Pan et al. 2014) and obesity (Sayon-Orea, Bes-Rastrollo et al. 2013); a mechanism through which associations with OA may be influenced.

With increased consumption of fried foods having been found to be associated with inflammatory disease, the intake of fats and the negative effect on inflammation is still a mechanism through which the association seen in this research could be occurring. There has been no previous research investigating the direct effect of fried food consumption on OA, so further studies are needed.

The results from the initial dietary analyses also showed some unexpected results. Leafy vegetables showed a positive association with knee OA ($p<0.001$) (Table 8) closely followed by the finding that cruciferous vegetables is positively associated with risk of hip pain ($p<0.001$) (Table 9) and knee pain ($p<0.001$) (Table 10). However, the consumption of the food group other vegetables is suggested to decrease the risk of knee OA ($p=0.023$) (Table 8).

An explanation as to the associations seen with the vegetable food groups could be that those with pain and suffering from OA (who were aware of their symptoms, and who attempted to alleviate these through self-help) began eating more vegetables due to the health messages through the media. As a result, when reporting dietary intake these participants would have reported high consumption of vegetables but would have also been diagnosed with clinical OA and or reported feeling pain, causing an association where there likely is not. These associations may also have been seen due to the inaccuracy of FFQ data, discussed further in Chapter 11. The finding that energy intake and the food group cruciferous vegetables are positively associated with hip pain explains less than 0.5% of the variation in hip pain.

In summary for non-dietary variables in both the case-control OA and musculoskeletal pain analysis, BMI was found to increase the risk of all OA and pain variables which explained a much higher percentage of the variation of the data.

4 A Gender-Specific Investigation into Dietary and Lifestyle Factors and Osteoarthritis/Pain of the Hip and Knee. A Case-Control Analysis using Data from EPIC-Norfolk.

4.1 Chapter Overview

The previous chapter investigated those factors that best predict clinical OA and joint pain. Due to EPIC-Norfolk containing both male and female participants, and with OA and joint pain being more prevalent in females, this analysis uses hip and knee pain data at the first health check (post baseline data) to investigate explanatory variables separately for males and females. With the female data, menopause, oral contraceptive and hormone replacement therapy status were all included. As menopause status and HRT use are age dependent, stratification by age quartiles took place for just the female data. The main finding from this chapter is that BMI is significantly associated with females in all age quartiles.

4.2 Introduction

Studies have shown that prevalence in chronic musculoskeletal pain can be explained by differences in gender, with females being at a greater risk (Wijnhoven, de Vet et al. 2006). It has been suggested that the onset of OA/pain could be related to an incidence of hormone imbalance (Spector and Campion 1989). Musculoskeletal pain is prevalent in females and onset is typically seen around the time of menopause (Prieto-Alhambra, Judge et al. 2014). Studies have found that postmenopausal women have significantly higher prevalence of musculoskeletal symptoms compared with premenopausal women. It is, therefore, important to investigate age related differences in musculoskeletal symptoms in females (Gao, Lin et al. 2013).

The EPIC-Norfolk cohort provides dietary, lifestyle and OA/pain data, for both males and females. This analysis will investigate those factors which are implicated in the development of joint pain, in males and females separately. With age being key to the development of joint pain particularly in females, the analysis in this chapter is stratified by age to investigate differences in those factors that best predict the onset of joint pain in females of different age groups.

4.3 Methods

4.3.1 Lifestyle and Dietary Variables

This chapter uses the independent variables and covariates which are described in Tables 4-6 in Chapter 3.3.4. Please see Chapter 3.3.3 for the methods used for the collection of dietary data and blood lipid data.

Lifestyle variables include age, BMI, gender, waist circumference, hip circumference, height, smoking status, teetotal, alcohol intake, ethnic group, physical activity, cholesterol, triglycerides, HDL and LDL. For females, menopause status, oral contraceptive use and

hormone replacement therapy use were all included also. Dietary variables of interest include citrus fruits, non-citrus fruits, leafy vegetables, other vegetables, allium vegetables, cruciferous vegetables, green vegetables, yellow vegetables, fatty fish, other fish, chicken, meat, meat products, eggs, cheese, brown bread, milk, snacks, chips and energy intake.

4.3.2 Musculoskeletal Pain Data

Pain data within EPIC-Norfolk was collected at multiple time points: 18 months post baseline (first health check), 3 years post baseline (second health check) and 10 years post baseline (third health check). Please see Figure 10, Chapter 3.2). The pain data used for the cross-sectional analysis was that of the first health check post baseline (please see Table 3, Chapter 3.3).

4.3.3 Statistical Analysis

The relationship between hip and knee pain and the independent variables (outlined previously) was investigated in EPIC-Norfolk by putting all variables into a logistic regression model. A stepwise algorithm was used to remove each that was non-significant so that only those independent variables that were significantly associated with hip and knee pain remained.

Data was used to generate four age quartiles for analysis. These were as follows:

- Lower quartile (39.6 to 51.6 years)
- Lower-middle quartile (51.6 to 59.6 years)
- Upper-middle quartile (59.6 to 67.5 years)
- Upper quartile (67.5 to 79.8 years).

4.4 EPIC-Norfolk Gender Analysis Results

4.4.1 Gender Analysis of Hip Pain in EPIC-Norfolk

Table 11 shows those explanatory factors that best predict hip pain, in males and females separately.

TABLE 11. SIGNIFICANT PREDICTORS OF HIP PAIN, IN MALES AND FEMALES, IN EPIC-NORFOLK.

Significant predictors of hip pain in males	**OR**	**95% CI**
Age	1.03	(1.02, 1.04)
BMI	1.08	(1.05, 1.10)
Brown bread	0.95	(0.91, 0.99)
Significant predictors of hip pain in females		
Age	1.03	(1.02, 1.04)
BMI	1.07	(1.05, 1.08)
Physical Activity	0.73	(0.60, 0.88)
HRT use	0.60	(0.52, 0.70)
Teetotal status	0.78	(0.68, 0.90)

The results from the analysis investigating hip pain in males found that increased age is suggested to increase the risk of hip pain ($p<0.0001$). Increased BMI is also suggested to increase the risk of hip pain ($p<0.0001$). Brown bread consumption was significantly negatively associated with hip pain ($p=0.022$). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

The results from the analysis of independent variables and hip pain in females found that increased age is significantly positively associated with increased risk of hip pain ($p<0.0001$). Not being teetotal is significantly associated with risk of hip pain ($p<0.001$). Physical activity is significantly associated with hip pain in females suggesting that an increase in physical activity decreases the risk of hip pain ($p=0.0026$). Never having used HRT is significantly associated and is suggested to decrease the risk of hip pain in comparison to being a current user of HRT ($p<0.0001$). Increased BMI is significantly associated with hip pain and is suggested to increase the risk of hip pain ($p<0.0001$). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.2 Hip pain in females in EPIC-Norfolk

A logistic regression analysis investigated associations between explanatory variables and females stratified into quartiles based on age. Table 12 shows those variables that best predict hip pain, in females, in each quartile.

TABLE 12. SIGNIFICANT PREDICTORS OF HIP PAIN, IN FEMALES, STRATIFIED BY AGE IN EPIC-NORFOLK.

Significant predictors of hip pain in females	**OR**	**95% CI**
Lower Quartile		
HRT use	0.55	(0.41, 0.74)
BMI	1.05	(1.02, 1.08)
Lower-middle Quartile		
HRT use	0.52	(0.40, 0.67)
BMI	1.09	(1.07, 1.12)
Upper-middle Quartile		
BMI	1.08	1.06, 1.11)
Upper Quartile		
Physical Activity	0.43	(0.25, 0.69)
BMI	1.05	(1.02, 1.08)

4.4.2.1 Lower Quartile

Analysis that took place with females, aged 39.6 to 51.6 years, showed that never having used HRT is significantly associated with hip pain in comparison to being a current user of HRT ($p<0.0001$). Increased BMI is suggested to increase the risk of hip pain ($p<0.001$). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.2.2 Lower-middle Quartile

Analysis that took place with females, aged 51.6 to 59.6 years, showed that never having used HRT is negatively associated with hip pain in comparison to being a current user of HRT ($p<0.0001$). Increased BMI is suggested to increase the risk of hip pain ($p<0.0001$). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.2.3 Upper-middle Quartile

Analysis that took place with females, aged 59.5 to 67.5 years, showed that BMI is significantly associated with hip pain and suggests an increased risk of hip pain ($p<0.0001$). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.2.4 Upper Quartile

Analysis that took place with females, aged 67.5 to 79.8 years, showed that physical activity is significantly negatively associated with hip pain ($p<0.001$). BMI is significantly positively associated with hip pain ($p<0.001$). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.3 Gender Analysis of Knee Pain in EPIC-Norfolk.

Table 13 shows those explanatory factors that best predict knee pain in males and females separately.

TABLE 13. SIGNIFICANT PREDICTORS OF KNEE PAIN, IN MALES AND FEMALES, IN EPIC-NORFOLK.

Significant predictors of knee pain in males	**OR**	**95% CI**
Age	1.02	(1.02, 1.03)
BMI	1.10	(1.08, 1.12)
Significant predictors of knee pain in females		
Age	1.04	(1.03, 1.04)
BMI	1.11	(1.10, 1.12)
HRT use	0.71	(0.62, 0.81)

The results from the analysis investigating knee pain in males showed that increased age is suggested to increase the risk of knee pain ($p<0.0001$). BMI is a significant explanatory suggesting that increased BMI also increases the risk of knee pain ($p<0.0001$). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

The results from the analysis investigating knee pain in females showed that increased age is suggested to increase the risk of knee pain ($p<0.0001$). HRT is a significant explanatory factor. Never having used HRT is negatively associated with knee pain ($p<0.0001$) in comparison to being a current user of HRT. Increased BMI is suggested to lead to increased

risk of knee pain (p<0.0001). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.4 Knee Pain in females in EPIC-Norfolk.

As with hip pain, analysis took place with females stratified into quartiles based on age. Table 14 shows those explanatory factors that best predict knee pain, in females, in each quartile.

TABLE 14. SIGNIFICANT PREDICTORS OF KNEE PAIN, IN FEMALES, STRATIFIED BY AGE IN EPIC-NORFOLK.

Significant predictors of knee pain in females	OR	95% CI
Lower Quartile		
HRT use	0.59	(0.45, 0.79)
BMI	1.07	(1.04, 1.10)
Triglycerides	1.30	(1.11, 1.52)
Lower-middle Quartile		
HRT use	0.69	(0.54, 0.87)
BMI	1.11	(1.09, 1.14)
Non-citrus fruits	1.55	(1.12, 2.18)
Upper-middle Quartile		
BMI	1.11	(1.09, 1.13)
Upper Quartile		
BMI	1.12	(1.10, 1.15)

4.4.4.1 Lower Quartile

Analysis that took place with females, aged 39.6 to 51.6 years, found that never having used HRT is negatively associated with the risk of knee pain (p<0.0001) in comparison to being a current user of HRT. Increased triglycerides is significantly positively associated with knee pain (p=0.001) and BMI is also significantly positively associated with knee pain (p<0.0001). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.4.2 Lower-middle Quartile

Analysis that took place with females, aged 51.6 to 59.6 years, found that never having used HRT is negatively associated with knee pain (p=0.003) in comparison to being a current user of HRT. Non-citrus fruit consumption is significantly positively associated with knee pain (p=0.002) and BMI is significantly associated with knee pain suggesting that increased BMI increases the risk of knee pain (p<0.0001). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.4.3 Upper-middle Quartile

Analysis that took place with females, aged 59.6 to 67.5 years, found that BMI is significantly associated with knee pain and suggests that increased BMI leads to an

increased risk of knee pain (p<0.0001). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.4.4.4 Upper Quartile

Analysis that took place with females, aged 67.5 to 79.8 years, found that BMI is significantly positively associated with knee pain (p<0.001). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

4.5 Discussion

The consistent factors across the gender analyses were the associations with age and BMI. BMI appeared consistently throughout the analysis and is a strong predictor of hip and knee pain in both males and females. It is not unexpected that BMI shows an increase in the risk of hip and knee pain as the joint is under pressure and could either be experiencing pain as a result of the excess weight or undergoing changes due to OA resulting in the pain experience. BMI was found to be positively associated with hip and knee pain in females in every quartile. This finding supports that of previous studies that found higher BMI to be associated with increased prevalence of knee pain in a cohort of women aged 35-64 years (Gao, Lin et al. 2013).

Physical activity was found to be inversely associated with hip pain in females (p=0.003). For management of OA, clinical guidelines recommend exercise which can be combined with pain relief and physiotherapy for treatment purposes (Bennell and Hinman 2011). A more recent study showed that higher levels of leisurely physical activity is associated with increased levels of tibiofemoral cartilage glycosaminoglycan in women who were postmenopausal and suffering from less severe knee OA (Munukka, Waller et al. 2017). Those studies investing physical activity as a preventative measure are inconclusive. No associations were found in the Framingham cohort when investigating the effects of exercise on older adults (Felson, Niu et al. 2007). Meta analyses have explored studies that have investigated the role of specific activities, such as running, on OA and have not been able to draw any conclusions (Timmins, Leech et al. 2017). The 1958 British birth cohort study also reported that higher levels of CWP remained when participants reported high or low levels of physical activity (VanDenKerkhof, Macdonald et al. 2011). Therefore inactivity and physical activity could both act as risk factors for pain. Those with low levels of physical activity become at high risk of developing chronic widespread pain. Those with higher levels of physical activity could be affected directly (where high levels of physical activity could lead to injury to the joints and muscles resulting in pain), or indirectly (where inactivity is the result of injury therefore putting them at risk) (VanDenKerkhof, Macdonald et al. 2011).

HRT use was suggested to be positively associated with hip and knee pain in females throughout the age dependent analysis. The results found that never having used HRT suggest a decrease in the risk of pain (Table 12 & 14). Hormone Replacement Therapy is a form of relief from menopausal symptoms and is found in this analysis to be positively associated with both hip and knee pain particularly in the lower to lower-middle quartiles (those aged 40-60 years). A large study, in the Framingham cohort, found the opposite effect of estrogen use when investigating radiographic OA in a cohort of 831 females

(Hannan, Felson et al. 1990). Aromatases synthesise oestrogen in connective tissue and oestrogen receptors are found in cartilage, subchondral bone and synovium. In vitro pro-chondrogenic effects have been seen from hormones such as oestrogen and progesterone which have been shown to inhibit IL-1 catabolic effects in cartilage. However, an increase in OA (and in the degradation of the matrix) is seen when pharmacological doses of oestrogen are administered. HRT (also known as MHT) alongside selective estrogen receptor modulators (SERMs) (such as levormeloxifene and raloxifene) have been shown to reduce levels of the two OA matrix degradation biomarkers COMP and CTXII. It is suggested that HRT could play a role in OA via its protective effects on bone changes (seen in the progression of OA) and also by inhibiting pain pathways as oestrogen is anti-nociceptive and a deficiency has been shown to be associated with increased pain (Watt 2016). This association could be one seen due to cross-sectional design. HRT is sought after because of musculoskeletal symptoms, so many of the epidemiological studies investigating this area are conflicted (Watt 2016) and poses the question of which direction the association occurs.

There were differences in the significant results for hip and knee pain between males and females. As well as age and BMI, an inverse association was seen between brown bread consumption and hip pain in males. Brown bread is considered a source of dietary fibre due to the presence of whole grains (Jones, Mann et al. 2017). As pain is known to be associated with OA (due to excess weight), this finding is of particular interest when taking into account previous research, which has found whole grain sources of fibre to be inversely associated with body composition (Gibson, Eriksen et al. 2019). A lower intake of bread and particularly whole grain bread has been reported in those who had central obesity in a study, involving both males and females. Another study also supports the association seen in this analysis in males, which found dietary fibre to be inversely associated with BMI only in males (Van de Vijver, Van den Bosch et al. 2009). A way in which pain in OA is influenced by diet could, therefore, be through the molecular mechanism that links diet, the gut microbiome and fat storage within adipose tissue.

The gut microbiota have been found to influence a number of metabolic processes including energy absorption, appetite and glucose and lipid metabolism (Chassaing and Gewirtz 2014). Short chain fatty acids (SCFAs) due to the fermentation of dietary fibre from bacteria in the gut microbiome have been shown to have an impact on metabolic pathways. The production of these SCFAs leads to the release of the appetite regulating hormones, peptide YY (PYY) and glucagon like peptide-1 (GLP-1). These are able to activate the G protein coupled free fatty acid receptor 2 (FFAR 2) found on colonic L cells (Tolhurst, Heffron et al. 2012) for which the SCFA with the highest affinity has been found to be propionate (Le Poul, Loison et al. 2003).

Propionate has been a focus of recent research and has been found to stimulate the release of hormones in vivo, reduce food intake and prevent weight gain in adults (Chambers, Viardot et al. 2015). The gut microbiota is closely linked to Metabolic syndrome of which obesity is one of the main features (Festi, Schiumerini et al. 2014). In smaller cohort studies component of metabolic syndrome have been found to be positively associated with OA (Askari, Ehrampoush et al. 2017) however in larger more sufficiently powered studies metabolic syndrome has not shown an association with OA (Niu, Clancy et al. 2017). Despite conflicting epidemiological evidence there is likely a very close link between diet, the gut microbiome, metabolic processes and OA/pain.

Non-citrus fruits were found to be positively associated with knee pain (p=0.002) in those aged 51.6 to 59.6 years. This is unexpected as were the vegetable food groups also showing positive associations with knee OA and hip and knee pain in Chapter 3. Again this could be due to the reliability of FFQ data or to the fact that participants were trying to self-help and had already modified their diet due to general health messages about fruit and vegetables being beneficial for health. This, as mentioned with the associations seen with the vegetable food groups in the last chapter, would result in the reporting of a high intake of fruit and vegetables as well having clinical OA diagnosis and pain symptoms which would explain the positive associations seen.

Alongside age and BMI, not being teetotal was found to be significantly associated with hip pain ($p<0.001$) suggesting, as seen previously, that being teetotal is not as beneficial as moderate alcohol intake, as discussed in Chapter 3. With the J and U shaped curves in mind, mechanisms by which excess alcohol consumption could be impacting pain is through direct interaction with cellular components and the effect of alcohol metabolism on the build-up of oxidative stress and inflammatory state.

Alcohol metabolism produces acetaldehyde and ROS resulting in oxidative stress and damage, with secondary stress resulting from a release of pro-inflammatory cytokines due to the cellular response to alcohol. Ethanol is able to interact with the cell membrane, modifying receptors alongside signalling proteins resulting in altered function and damage to healthy tissues (Jung, Callaci et al. 2011). Excessive alcohol consumption leads to increased ethanol metabolism and has been found to be associated with high levels of circulatory pro-inflammatory mediators (McClain, Barve et al. 1999).

Alcohol consumption has been found to be associated with severe OA of the DIP and PIP joints of the hand and although this study found no statistical significance in the results between gender, this has previously appeared to more robust in females than males (Haugen, Magnusson et al. 2017). The effects of alcohol consumption may also depend on the type of alcoholic drink consumed. Consumption of beer has been found to be positively associated with hip and knee OA. However, wine has been found to be inversely associated with knee OA and spirits have been found to be inversely associated with hip OA in a dose dependent manner (Muthuri, Zhang et al. 2015). Not only can excess alcohol consumption be a factor that increases risk of OA/pain, it is also a way of self-medicating in those suffering with arthritic symptoms. This has been noted as so excessive that clinical studies have found, that for 28% of patients with chronic pain, alcohol becomes an abusive substance (Riley III and King 2009) leading to uncertainty about causality.

5 Exploring Dietary Patterns and Radiographic OA of the Hip, Knee and Hand. A Cross-Sectional Analysis using Data from TWINS-UK.

5.1 Chapter Overview

Having discussed the current evidence for the role of nutrition in OA and with the nutrition related variables showing food groups high in fats to be positively associated with hip and knee OA in EPIC-Norfolk, it is important to further investigate and add to these findings. This chapter investigates cross-sectional associations between PCA derived dietary patterns, collected from FFQs, and Radiographic OA variables, to assess the risk that different dietary patterns may have on hip, knee and hand OA in TWINS-UK. The main finding from this cross-sectional analysis is that the 'Traditional English' dietary pattern is significantly positively associated hand OA.

5.2 Introduction

The previous two chapters have investigated lifestyle and dietary factors in relation to hip and knee OA and hip and knee pain. The nutritional analysis has found that food high in fats are positively associated with hip and knee OA but there have also been some unexpected results.

TWINS-UK, although smaller in size when compared to the EPIC-Norfolk cohort, has a wealth of nutritional data available. In addition to hip and knee OA, there also exists hand OA. Despite the prevalence of hand OA being higher than that of other joint sites, knee OA has been the focus of research, with very little if any nutritional research investigating the effects of diet on hip and hand OA. Previous studies have investigated dietary modification to reduce occurrence of disease. This is due to pharmacologic treatment of arthritic symptoms leading to other chronic health issues. Research has indicated that diet can improve inflammation and symptoms of OA, without negative side effects, and there are many inflammatory pathways and biological mechanisms by which diet may be having an effect on OA, as discussed in Chapter 1.

Although there are previous studies that have investigated the associations between diet and OA, most seems to have focused on how diet affects inflammation as opposed to associations with OA and joint pain. The purpose of this research, therefore, is to investigate the effects of different dietary patterns on radiographic hip, knee and hand OA in an all-female cohort, which was obtained from the TWINS-UK Registry.

5.3 Methods

5.3.1 Food Frequency Questionnaires and Principal Component Analysis

From the TWINS-UK FFQs, the recorded data initially consisted of information reported on the 131 food items. Participants were asked how often they consumed each food, on average, within the last year of which the options were:

- Never or less than once per month
- 1-3 times per month
- Once a week
- 2-4 times per week
- 5-6 times per week
- Once a day
- 2-3 times per day
- 4-5 times per day
- 6 or more times per day

Participants were also asked not to leave any questions within the FFQ blank and to estimate as best they could if they were not sure. The food items data recorded was then gathered into 54 food groups based on similarities in nutrient content and use when cooking. Food items were also combined, for heritability analysis (Teucher, Skinner et al. 2007), to create 24 custom food types based on nutrient content and common tastes: Fruit and Vegetable Sources, Garlic, Alcohol, Fruit, Coffee, Red Meat, Fish (other than fried), Tea, Root vegetables, Allium, Brassica, Low fat dairy/dieting, Wholemeal grains, Salad, Nuts, Sweet tastes, Legumes-earthy, Eggs, Savoury tastes, Poultry, Refined grains, Fast food, Fresh Juice and High-fat dairy.

Also, from the FFQ data, there were derived five diverse patterns of dietary consumption which were identified through principal component analysis (PCA) (Teucher, Skinner et al. 2007), using the energy adjusted frequency of intake (number of servings per week), and named according to what was typically seen within each pattern:

- Fruit and Vegetable (“Frequent intakes of fruit, allium and cruciferous vegetables; low intakes of fried potatoes”)
- High Alcohol (“Frequent intakes of beer, wine and allium vegetables; low intakes of high fibre breakfast cereals and fruit”)
- Traditional English (“Frequent intakes of fried fish and potatoes, meats, savoury pies and cruciferous vegetables”)
- Dieting (“Frequent intakes of low fat dairy products, low-sugar soda; low intake of butter and sweet baked products”)
- Low Meat (“Frequent intakes of baked beans, pizza and soy foods; low intakes of meat, other fish and seafood and poultry”).

The five distinct dietary patterns used in this analysis are therefore Fruit and Vegetable, High Alcohol, Traditional English, Dieting and Low Meat (Teucher, Skinner et al. 2007).

Please see Appendix 2 for the distribution of food groups and FFQ items from which these five dietary patterns were derived.

5.3.2 Radiographic OA Data

Within TWINS-UK there is a large volume of radiographic OA data from those participants that suffered from OA. The severity of OA was assessed using the Kellgren Lawrence grading system. This grading system assesses the severity of OA based on specific changes that occur within the joint. These radiological features include the following:

- Osteophyte formation on the joint margins or tibial spines
- Periarticular ossification
- Narrowing of joint space and therefore loss of cartilage which is connected with sclerosis of the layer of subchondral bone beneath the cartilage
- Within the layer of subchondral bone there can be seen small areas that contain pseudocysts with sclerotic walls surrounding these areas
- Changes in the shape of the ends of the bone especially within the head of the femur

Based on the above OA, when assessed through looking at x-rays, could then be given a grade: 0 (none), 1 (doubtful), 2 (minimal), 3 (moderate) and 4 (severe) where grade 0 represents the definite absence of changes, grade 2 represents a definite presence but with a low level of severity and grade 4 represents chronic OA (Kellgren and Lawrence 1957).

Table 15, below, shows the numbers of those within the TWINS-UK cohort that have presence or absence of knee, hip and hand OA, based on the above Kellgren Lawrence grading system, and also shows the number of those without X-ray data.

TABLE 15. CHARACTERISTICS OF DEPENDENT VARIABLES OF INTEREST WITHIN TWINS-UK.

	Present	Absent	No. without X-ray data
Knee OA	355	1297	6699
Hip OA	102	1198	7051
Hand OA	291	1560	6500

5.3.3 Energy Intake and Confounding Factors

Analysis of the nutritional data, from the TWINS UK FFQs, adjusted for total energy intake and other common confounders.

The models used firstly, adjust for total energy intake and secondly, for energy intake as well as other confounders; age, BMI, physical activity, smoking history, alcohol consumption and the use of Hormone Replacement Therapy which have all been suggested to have relevance in the development of chronic OA. The models of adjustment within this cross-sectional analysis are therefore:

- Model 1. Adjustment for energy intake.
- Model 2. Adjustment for energy intake, age, BMI, smoking, alcohol consumption, physical activity and HRT.

Gender was not adjusted for in the TWINS-UK analysis, due to it being an all-female subset of data from the cohort.

TABLE 16. CHARACTERISTICS OF CONFOUNDERS WITHIN TWINS-UK DATA.

Total	8351			**No. without X-ray data**
Mean age (years)	52.19 (SD 14.25)			6466
Mean BMI (kg/m²)	25.18 (SD 4.63)			6490
	Active	**Inactive**		
Physical Activity	179	1659		6513
	Current	**Social**	**Never**	
Alcohol	521	539	200	6604
	Current use	**Former-use**	**Never use**	
Smoking	345	534	939	6533
HRT	416	248	1155	6527

As can be seen from Table 16, above, the total number of participants is lower in TWINS-UK than in EPIC-Norfolk and comprises an all-female cohort. The mean age of this cohort is less than in EPIC-Norfolk but is still the age at which onset and progression of OA and associated pain is typically seen. The mean BMI in TWINS-UK is also similar to that of EPIC-Norfolk.

In TWINS-UK, age, BMI and energy intake are all higher in OA cases than non-cases as overall the TWINS-UK cohort is characterised by higher levels of inactivity, more alcohol consumption and less smoking and HRT use which is reflected across the OA variables for both cases and non-cases (see Table 17). However the exception, when investigating the data specifically for knee, hip and hand OA, is that participants have higher levels of activity across cases and non-cases.

TABLE 17. CHARACTERISTICS OF TWINS-UK DATA BY OA SITE.

	OA Cases				**Non cases**			
Knee OA	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	59	39	79	8	53	24	76	7
BMI (kg/m²)	26.5	16.2	44.0	4.6	24.4	16.2	51.4	4.0
Energy Intake (kcal)	2004.83	730.39	3700.19	505.94	1981.47	794.07	3885.62	476.79
Fruit and Vegetable	0.43	-3.70	14.51	2.11	0.23	-5.64	13.28	1.89
High Alcohol	-0.45	-4.36	3.81	1.35	-0.20	-4.38	4.99	1.37
Traditional English	0.38	-4.33	9.24	1.44	0.11	-4.17	6.36	1.33
Dieting	0.37	-3.77	4.49	1.23	0.27	-4.00	7.50	1.15
Low Meat	-0.31	-3.17	4.45	1.08	-0.21	-3.82	6.97	1.07
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	188	125			748	375		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	176	99	43		708	353	128	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	53	97	188		244	376	626	
HRT	70	56	217		306	148	786	
Hip OA	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	58	42	73	8	53	24	73	8
BMI (kg/m²)	25.8	16.2	40.4	4.5	24.8	16.2	48.2	4.3
Energy Intake (kcal)	2040.79	1070.50	3276.11	476.84	1986.89	737.44	3885.62	490.24
Fruit and Vegetable	-0.02	-5.64	6.71	2.05	0.33	-4.57	14.51	1.97
High Alcohol	-0.55	-4.36	4.60	1.44	-0.13	-3.96	4.70	1.36
Traditional English	0.31	-3.14	3.76	1.27	0.13	-4.33	9.24	1.37

Dieting	0.34	-2.79	3.42	1.11	0.24	-4.00	7.50	1.21
Low Meat	-0.14	-2.94	2.89	1.10	-0.23	-3.82	6.97	1.09
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	47	40			662	367		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	47	30	19		682	328	104	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	21	26	53		231	357	590	
HRT	18	15	66		310	139	717	
Hand OA	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	62	45	79	6	53	24	76	7
BMI (kg/m²)	25.0	16.2	44.0	3.9	24.8	15.0	51.4	4.3
Energy Intake (kcal)	2027.63	737.44	3226.89	457.85	1972.68	730.39	3885.62	487.94
Fruit and Vegetable	0.23	-3.70	7.98	1.89	0.30	-5.64	14.51	1.96
High Alcohol	-0.55	-4.42	4.99	1.33	-0.19	-4.38	4.70	1.35
Traditional English	0.40	-2.93	4.19	1.26	0.15	-4.33	9.24	1.37
Dieting	0.51	-2.88	4.42	1.18	0.25	-4.00	7.50	1.17
Low Meat	-0.33	-3.38	4.19	1.07	-0.22	-3.82	6.97	1.07
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	159	86			891	478		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	125	97	25		852	423	166	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	39	89	147		289	433	760	
HRT	52	39	185		358	197	927	

5.3.4 Statistical Analyses

The TWINS-UK data was analysed using a multi-level model in order to take into account the twin pairs. A mixed effects logistic regression was used, with the models of adjustment outlined, to investigate cross-sectional associations between dietary pattern scores and the presence of radiographic OA in the hip, knee and hand. The statistical software programme Stata, version 12, was used for analysis and $p<0.05$ indicated statistically significant findings.

5.4 TWINS-UK Dietary Pattern and OA Results

A logistic regression analysis of the five derived dietary patterns and radiographic OA variables of interest (knee, hip and hand OA) took place, using the two models of adjustment outlined previously.

TABLE 18. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND RADIOGRAPHIC HIP OA IN TWINS-UK.

Hip OA	**Model 1 (n=1102)**		**Model 2 (n=626)**	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	0.88	(0.75, 1.02)	0.84	(0.68, 1.03)
High Alcohol	0.76	(0.61, 0.94)*	0.94	(0.69, 1.30)
Traditional English	1.12	(0.92, 1.37)	1.06	(0.82, 1.37)
Dieting	1.11	(0.88, 1.40)	1.09	(0.80, 1.49)
Low Meat	1.15	(0.89, 1.50)	1.11	(0.77, 1.60)

*p<0.05
**p<0.01

TABLE 19. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND RADIOGRAPHIC KNEE OA IN TWINS-UK.

Knee OA	**Model 1 (n=1417)**		**Model 2 (=800)**	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	1.07	(0.98, 1.17)	1.19	(1.06, 1.34)**
High Alcohol	0.84	(0.73, 0.96)	0.99	(0.81, 1.20)
Traditional English	1.21	(1.06, 1.38)	1.15	(0.97, 1.36)
Dieting	1.09	(0.94, 1.26)	1.11	(0.90, 1.36)
Low Meat	0.93	(0.79, 1.10)	0.84	(0.67, 1.07)

*p<0.05
**p<0.01

TABLE 20. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND RADIOGRAPHIC HAND OA IN TWINS-UK.

Hand OA	**Model 1 (n=1592)**		**Model 2 (n=901)**	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	1.00	(0.90, 1.13)	0.92	(0.79, 1.07)
High Alcohol	0.78	(0.66, 0.92)**	1.00	(0.78, 1.29)
Traditional English	1.22	(1.04, 1.42)*	1.28	(1.03, 1.58)*
Dieting	1.24	(1.04, 1.49)*	1.09	(0.85, 1.40)
Low Meat	0.84	(0.68, 1.03)	0.95	(0.70, 1.27)

*p<0.05
**p<0.01

The 'Fruit and Vegetable' dietary pattern shows a significant positive association (p=0.004) with knee OA (Table 19) when adjusted for confounding factors. An initial association (p=0.012) was observed between the 'High Alcohol' dietary pattern and hip OA (Table 18), which disappeared after adjusting for confounding factors. The 'Traditional English' dietary pattern shows a consistent statistically significant positive association (p=0.015) with hand OA (Table 20), which was still seen after adjustment of confounding factors (p=0.025). The 'Dieting' pattern shows a significant positive association (p=0.018) with hand OA initially, and the 'High Alcohol' dietary pattern shows an association with hand OA (p=0.004) (Table 20) but both of these associations disappeared when adjusting for confounding factors.

5.5 Discussion

A westernized dietary pattern such as the typical English diet, represented by the 'Traditional English' dietary pattern, contains elevated amounts of highly processed meals and unhealthy snacks alongside a lack of fresh foods. This so called 'fatty' diet (due to the saturated fat content of the foods consumed e.g. meats, processed meat products and animal fats (Landaeta-Diaz, Fernandez et al. 2013)) results in a higher dietary intake of fatty acids. The effects of these nutrients in high volumes are widely known to have negative effects on health.

When investigating OA it is important to consider the whole diet effect on the disease process, however, consideration must also be given to nutrients and compounds found within this dietary pattern, in order to further investigate the biological mechanisms that may be playing a role in the development of the disease. Reviewing the key results it can be seen that a higher intake of the 'Traditional English' dietary pattern is suggested to increase the risk of hand OA by 28% (p=0.025). The concept in epidemiological research of association versus causation is discussed further in Chapter 10. The discussion below outlines how a diet high in fats could be negatively impacting OA, in terms of the mechanisms of action.

Whilst dietary fats and lipids, are incredibly important, due to their nature as an efficient energy source and also the role that they play in cell structure and cellular signalling, they can also be a contributing factor to detrimental metabolic outcomes. With an increased

intake of lipids, the lipid influx into the adipose tissue is exceeded, resulting in harmful lipids species accumulating in cells and in the circulation. Lipotoxicity can influence lipid metabolism, which is closely linked to immune response through the activation of signalling intermediates, and can induce chronic low grade inflammation (Ertunc and Hotamisligil 2016). Previous studies, investigating the role of fatty acids in OA, have indicated the detrimental effect of saturated fatty acids and ω-6 PUFA on chondrocytes. This occurs via induction of prostaglandins and upregulation of gene expression related to apoptosis and cartilage degradation (Ertunc and Hotamisligil 2016).

Exogenous saturated fatty acids, such as palmitate, have been investigated for their pro-inflammatory effects. The mechanisms of excess lipids are related to the properties of cellular organelles such as the endoplasmic reticulum (ER) (Pineau, Colas et al. 2009). Palmitate is taken up by cells, which upregulates cytosolic small nucleolar RNAs (snoRNAs) involved in ER stress (Michel, Holley et al. 2011). Protein Kinase R (PKR), involved in palmitate-mediated snoRNA upregulation, can also activate protein complexes (known as inflammasomes which initiate inflammatory responses), JNK and AP-1 mediated inflammatory gene expression (Nakamura, Furuhashi et al. 2010).

Palmitate supports the synthesis of diacylglycerols which, in turn, activate stress kinases (JNK and p38) and NF-κB (Glass and Olefsky 2012). Palmitate supports synthesis of ceramides which activates JNK signalling and can also activate toll-like receptor 4 (TLR4) signalling (Lee, Sohn et al. 2001). This leads to activation of inflammasomes and stimulation of transcription factors such as NF-κB and AP-1 (Kang and Tang 2012). Figure 11 shows the signalling pathways influenced by intake of this saturated fatty acid. This figure also shows how cholesterol is taken up by cells (through scavenger receptors) and accumulation, as both oxidized cholesterol and cholesterol crystals, is able to activate TLR4 and stress kinases in addition to inflammasome activation and pro-inflammatory gene expression. Another negative effect of lipotoxicity is the production of ROS from the mitochondria which can also result in inflammasome activation (Ertunc and Hotamisligil 2016).

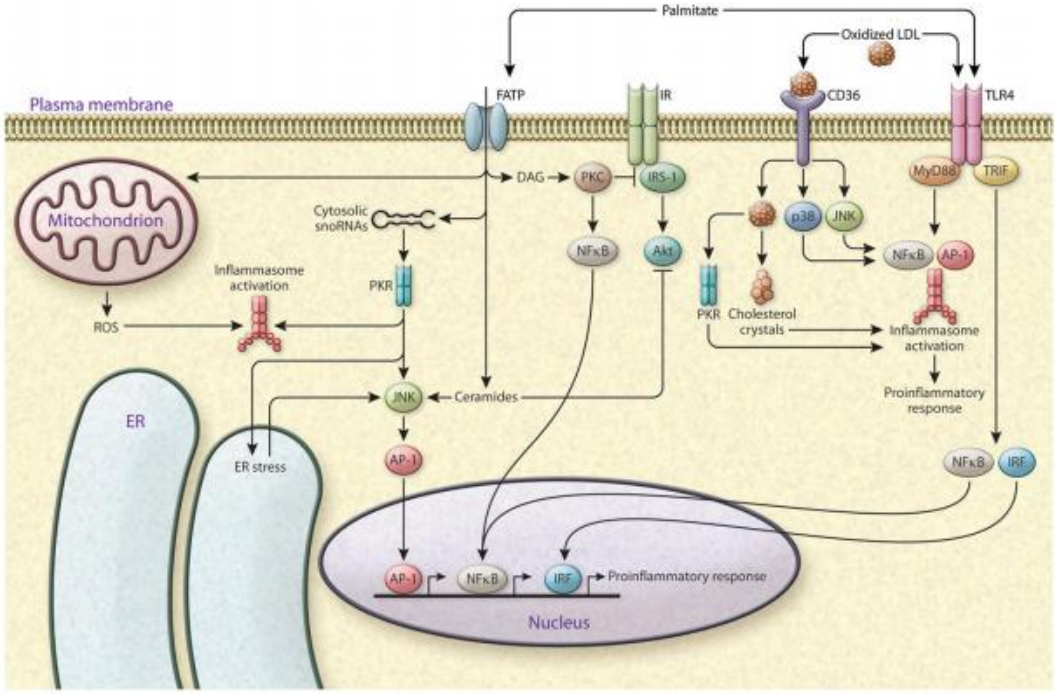

FIGURE 11. THE MECHANISMS OF ACTION OF LIPIDS INVOLVING THE ENDOPLASMIC RETICULUM (PINEAU, COLAS ET AL. 2009).

ω-6 PUFAs exist excessively within the western diet at a much higher ratio to ω-3 PUFAs (15:1) than the recommended range (from 2:1 to 5:1) (Simopoulos 2006). High levels of ω-6 PUFAs are known to be associated with inflammation (Tokuyama and Nakamoto 2011). A mechanism of action by which ω-6 PUFAs have an effect on inflammation is the generation of prostaglandins, derived from the ω-6 PUFA arachidonic acid which is metabolised by COX-1 and COX-2 after AA is released from the plasma membrane by phospholipases. There are four prostaglandins which are vital to the inflammatory process due to their ability to behave as inflammatory mediators and play a crucial role in a number of signalling pathways (Ricciotti and FitzGerald 2011). Other derivatives from ω-6 PUFAs are inflammatory eicosanoids and inflammatory cytokines, such as IL-1 and IL-6 (Tokuyama and Nakamoto 2011), known to play a role in the development of inflammatory joint disease.

The effects of fatty acids have been investigated extensively. Bone has an important role to play in the progression of Osteoarthritis (Lajeunesse and Reboul 2007). BMLs specifically have been associated with higher intakes of monounsaturated fatty acids and ω-6 PUFAs (Wang, Wluka et al. 2008). BML size has been found to be associated with HDL cholesterol (Doré, de Hoog et al. 2012).

Obesity is a known risk factor, not only for OA of weight baring joints but also for hand OA, (Yusuf, Nelissen et al. 2010) and it is clear that metabolic factors are involved in the progression of hand OA (Visser, De Mutsert et al. 2015). With a mean cohort BMI of 25.18kg/m² (and therefore the average BMI of the cohort being just within the overweight category) it is possible that excess adipose tissue (accumulated as a result of a 'Traditional English' dietary pattern) could be an underlying mechanism for increased risk of hand OA and could be influencing hand OA in different ways.

An increase in ingested fats, as part of the diet, are absorbed in the form of triglycerides and are transported and stored in adipose tissue. Adipose tissue then releases adipokines known to play a role in inflammation and there are many factors released as a result of excessive adipose tissue. Leptin, visfatin, adiponectin and resistin, are all involved in cartilage damage during the development of OA (Gabay and Gabay 2013) but further research is needed to understand the exact role as there is an abundance of conflicting evidence (Hu, Bao et al. 2011).

Leptin has been shown to increase risk of OA, by being associated with metabolic abnormalities, and shows a correlation with BMI. Despite an increase in leptin levels seen in OA, and correlations with cartilage destruction and osteophytes, its role is still uncertain. High leptin levels may be protective against cartilage degradation due to it stimulating chondrocyte's anabolic properties. Exogenous leptin also increases IGF-1 and TGFβ production. However, both leptin and its receptor (Ob-R) have been found to share common structural and functional properties with IL-6 and has, as a result, been classified as a cytokine. Upon binding to Ob-R, leptin has been found to trigger JAK/STAT as well as/PI3K/Akt/NF-κB and p300 signalling cascades (Tang, Lu et al. 2007).

Serum resistin levels have been found to be associated with radiographic changes, particularly subchondral bone, in hand OA. The pro-inflammatory nature of resistin appears to have been stopped by an NF-κB inhibitor suggesting a role of the NF-κB pathway in inflammation. Increased serum adiponectin has been found to be associated (in females) with erosive hand OA. A study that looked into the serum levels of adipokines and radiographic progression of hand OA, over 6 years, found that higher levels of adiponectin were associated with lower risk of the progression of hand OA. This particular adipokine has been shown to protect against cartilage damage (Yusuf, Ioan-Facsinay et al. 2011). A potential mechanism, by which this is thought to be possible, is adiponectin inducing tissue inhibitor of metalloproteinase-2 which reduces the defects in cartilage caused by MMPs (Chen, Chen et al. 2006).

Visfatin is also known to stimulate inflammatory mediators. Visfatin synthesis has been shown to be increased by IL-1β treatment in chondrocytes, in vitro. It has also shown a pro-degradative effect by increased synthesis and release of key matrix degrading enzymes MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 and has also been found to decrease aggrecan production in chondrocytes (Gosset, Berenbaum et al. 2008). Whilst many of these adipocytokines have shown catabolic functions in cartilage, there are still many conflicting studies. What is clear however, is the important role that adipose tissue plays, with respect to the pathophysiology of OA, through the release of these factors.

The finding that consumption of the 'Low Meat' dietary pattern is inversely associated with the risk of knee OA (Table 19) is intriguing. This is an aspect of the Mediterranean style diet and would be of interest in further analysis investigating the effects of a Med diet on OA. Another aspect of the Med diet is high fruit and vegetable content which, in this TWINS-UK analysis, shows unexpected associations. Findings indicate that at a higher intake of the 'Fruit and Vegetable' dietary pattern was suggested to increase the risk of knee OA by 19% ($p=0.004$). As discussed in Chapters 3 and 4, a reason for this unexpected result in the analysis could be that participants, who had severe OA and pain, had received advice and had an awareness of health messages about consumption of fruit and vegetables. They could, therefore, have already been self-treating through the modification of diet and this

would have, therefore, resulted in the reporting of eating lots of fruit and vegetables with high scores of OA/pain revealing the associations observed.

Whilst a diet high in fatty acids, as discussed, is a possible mechanism of action for influencing OA, another explanation could be due to a lack of nutrients, such as ω-3 fatty acids. These are known to be characteristic of a Mediterranean style dietary pattern and which have been widely investigated for their beneficial effects on health. Whilst there are mixed results for the 'low meat' and 'fruit and vegetable' dietary patterns, worth noting is the suggested protective effects of both of these dietary patterns with hand OA (Table 20). Although these associations are not statistically significant, they are observations consistent with the consumption of a Mediterranean dietary pattern. More research is, therefore, needed into the effects of a Mediterranean style diet on OA, specifically hand OA.

6 Exploring Dietary Patterns and Musculoskeletal Pain/Chronic Widespread Pain. A Cross-Sectional Analysis using Data from TWINS-UK.

6.1 Chapter Overview

Having investigated the associations between dietary patterns and radiographic OA, this chapter investigates the cross-sectional associations between dietary patterns and musculoskeletal pain in the TWINS-UK cohort. In this analysis musculoskeletal pain is used as a proxy for OA, due to it being one of the main symptoms of the disease. A logistic regression, with two models of adjustment, was used in order to assess the effect that dietary patterns have on both site specific pain and CWP, to see if there were any similar results to those dietary associations seen with radiographic OA. The main finding from analysis is that the 'Traditional English' dietary pattern was positively associated with pain variables but only reached statistical significance when investigating CWP.

6.2 Introduction

As discussed the relationship between OA and pain is key when defining OA. With the symptom of pain in the joint being a critical factor in determining clinical OA, the relationship between dietary factors and joint pain is of interest when considering the effect dietary factors may be having on OA.

Musculoskeletal pain affects over 50% of adult populations, is most prevalent in females and most commonly occurs at the knee, hand, hip, spine and shoulder (Watt 2018). Available in the TWINS-UK data set are four out of these five with more pain data in addition. Spine is represented by back pain and shoulder pain is combined with neck pain. TWINS-UK also provide foot pain and CWP for this analysis investigating the effects of dietary factors on pain. Joint pain acts as a proxy for OA, being one of the main symptoms of the disease, and due to the nature of pain being very complex and the theory of convergence pain, CWP acts as a proxy for joint pain.

The previous chapter suggested that a 'Traditional English' dietary pattern is positively associated with radiographic hand OA. This analysis will investigate associations between the PCA derived dietary patterns and the joint specific pain outcome variables (knee, hand, back, neck and shoulder, elbow and forearm, foot pain and CWP).

6.3 Methods

6.3.1 Principal Component Analysis

The PCA derived dietary patterns, discussed in Chapter 5, were again used in this analysis. Please refer to Chapter 5.3.2.

6.3.2 Pain Data

The site-specific pain data was collected through questionnaires which was sent to study participants asking subjects 'if they had experienced pain for the most part of the last month in the neck and back, or at the elbow, knee, thigh, hand or foot'. 57% of the 9036 twins responded to the questionnaire. The sample with musculoskeletal pain data contained 991 complete monozygotic twins (age range 18-82 years) with a mean age of 50.4 years and 1074 dizygotic twins (age range 19-82 years) with a mean age of 50.7 years. 18% of these respondents had radiographic OA data recorded and had been included in previous OA studies. The chronic widespread pain data was collected through two questionnaires completed in 2002 and 2008. Chronic widespread pain was defined in both the 2002 and 2008 questionnaires as having had pain in muscles, bones or joints lasting at least one week within the past three months.

TABLE 21. CHARACTERISTICS OF DEPENDENT VARIABLES OF INTEREST WITHIN TWINS-UK.

	Present	Absent	No. without pain data
Knee Pain	1876	3136	3339
Hand Pain	1369	3621	3361
Neck and Shoulder Pain	2370	2714	3267
Elbow and Forearm Pain	938	4020	3393
Foot Pain	1162	3660	3529
Back pain	372	1910	6069
CWP	1230	4347	2774

Across the musculoskeletal pain variables used in this chapter, age BMI and energy intake are all higher in cases than in non-cases with the exception of energy intake which was higher in non-cases for hand pain and CWP and age which was higher for non-cases of elbow and forearm pain (see Table 22).

Levels of physical activity but also alcohol consumption were reported to be higher across both cases and non-cases for all pain variables. Across all pain variables there were more participants that recorded never smoking compared to those that were current or former smokers and this was also the case with HTR use. Figures for those that had never used HRT were higher than those who were using or who had previously used HRT across cases and non-cases for all pain variables (see Table 22).

TABLE 22. CHARACTERISTICS OF TWINS-UK DATA BY MUSCULOSKELETAL SITE PAIN.

	Pain Cases				**Non cases**			
Knee Pain	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	55	32	79	7	53	32	76	8
BMI (kg/m²)	25.4	15.0	48.2	4.5	24.2	16.4	38.5	3.8
Energy Intake (kcal)	1955.86	626.32	3752.07	508.23	1931.94	676.18	3933.62	496.91
Fruit and Vegetable	0.48	-5.64	17.64	2.08	0.35	-7.45	14.51	2.04
High Alcohol	-0.13	-4.82	6.56	1.33	0.19	-6.86	7.40	1.43
Traditional English	0.28	-5.18	10.02	1.38	0.05	-4.66	9.24	1.34
Dieting	0.21	-4.66	5.23	1.24	0.18	-6.35	5.44	1.20
Low Meat	-0.18	-3.54	5.96	1.12	-0.09	-7.68	9.45	1.19
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	953	472			1456	815		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	270	163	69		371	183	59	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	84	156	271		95	177	352	
HRT	128	90	300		138	68	415	
Hand Pain	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	54	32	72	7	54	32	79	8
BMI (kg/m²)	25.1	15.0	45.4	4.4	24.6	16.2	48.2	4.0
Energy Intake (kcal)	1938.00	697.93	3752.07	499.67	1943.75	626.32	3933.62	501.34
Fruit and Vegetable	0.56	-7.45	11.18	2.00	0.34	-6.90	17.64	2.08

High Alcohol	-0.07	-4.36	7.40	1.36	0.11	-4.82	7.06	1.42
Traditional English	0.27	-4.33	6.33	1.36	0.09	-5.18	10.02	1.36
Dieting	0.19	-6.35	4.93	1.22	0.19	-5.93	5.44	1.21
Low Meat	-0.20	-7.68	5.17	1.13	-0.10	-5.65	9.45	1.17
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	706	374			1695	906		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	211	130	58		425	214	69	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	63	126	221		114	210	402	
HRT	124	68	222		140	91	497	
Neck and Shoulder Pain	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	SD
Age (years)	54	32	79	8	54	32	76	7
BMI (kg/m²)	24.9	15.0	48.2	4.4	24.7	16.4	46.0	3.9
Energy Intake (kcal)	1942.24	626.32	3933.62	501.19	1939.49	697.93	3885.62	500.40
Fruit and Vegetable	0.43	-7.45	17.64	2.06	0.37	-6.90	11.18	2.05
High Alcohol	0.04	-6.86	6.13	1.37	0.08	-4.38	7.40	1.43
Traditional English	0.22	-4.33	10.02	1.42	0.08	-4.66	7.98	1.30
Dieting	0.22	-6.35	5.44	1.25	0.17	-4.59	5.23	1.17
Low Meat	-0.12	-3.55	9.45	1.17	-0.13	-7.68	9.45	1.17
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	1143	619			1303	685		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	313	162	78		356	186	51	

	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	95	174	292		89	170	346	
HRT	156	89	317		114	74	421	
Elbow and Forearm Pain	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	53	34	79	7	54	32	79	8
BMI (kg/m²)	25.0	17.5	45.4	4.3	24.7	15.0	48.2	4.2
Energy Intake (kcal)	1946.83	626.32	3675.75	504.22	1935.40	676.18	3933.62	496.93
Fruit and Vegetable	0.41	-7.45	9.14	2.02	0.40	-6.90	17.64	2.06
High Alcohol	-0.04	-6.86	7.40	1.46	0.09	-4.82	7.25	1.39
Traditional English	0.28	-3.39	5.14	1.38	0.11	-5.18	10.02	1.36
Dieting	0.25	-5.93	5.23	1.28	0.18	-6.35	5.44	1.19
Low Meat	-0.17	-4.78	9.45	1.21	-0.12	-7.68	9.45	1.16
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	481	254			1905	1019		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	128	73	35		507	261	89	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	51	73	113		127	252	495	
HRT	72	35	132		187	117	574	
Foot Pain	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	54	32	72	7	54	32	79	8
BMI (kg/m²)	25.6	17.5	48.2	4.5	24.4	15.0	46.0	4.1
Energy Intake (kcal)	1962.21	697.93	3662.71	501.95	1932.84	626.32	3933.62	495.95

Fruit and Vegetable	0.48	-7.45	11.18	2.03	0.40	-6.73	17.64	2.07
High Alcohol	-0.09	-6.86	6.56	1.37	0.11	-4.82	7.40	1.41
Traditional English	0.29	-4.17	6.36	1.42	0.09	-5.18	10.02	1.34
Dieting	0.30	-6.35	5.44	1.27	0.16	-5.93	5.23	1.18
Low Meat	-0.19	-5.65	5.00	1.15	-0.11	-7.68	9.45	1.16
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	1069	578			1752	927		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	345	175	75		431	236	82	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	107	183	315		109	225	426	
HRT	169	105	337		162	92	509	
Back Pain	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	52	20	74	10	50	19	85	12
BMI (kg/m²)	26.0	16.2	52.4	5.0	24.7	13.9	51.7	4.3
Energy Intake (kcal)	1992.85	751.46	3752.07	516.36	1968.32	716.68	3885.62	488.64
Fruit and Vegetable	0.47	-5.64	8.91	1.95	0.19	-5.21	17.64	1.94
High Alcohol	-0.06	-4.08	6.12	1.36	0.01	-4.42	6.13	1.35
Traditional English	0.02	-5.70	4.16	1.27	0.07	-4.33	10.02	1.27
Dieting	0.17	-4.66	4.42	1.31	0.19	-6.35	5.23	1.19
Low Meat	-0.17	-3.31	4.74	1.14	-0.18	-5.65	5.96	1.10
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	304	170			988	543		

	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	126	58	21		475	251	90	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	49	65	95		142	237	443	
HRT	52	40	116		167	112	547	
CWP	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Age (years)	56	19	84	12	54	34	76	7
BMI (kg/m²)	26.7	15.1	51.4	5.2	24.1	17.1	46.0	3.8
Energy Intake (kcal)	1918.15	783.21	3488.61	493.55	1925.84	676.18	3933.62	499.34
Fruit and Vegetable	0.52	-7.45	11.18	2.07	0.40	-6.90	17.64	2.05
High Alcohol	-0.08	-6.86	5.01	1.34	0.12	-4.82	6.13	1.38
Traditional English	0.35	-5.18	6.33	1.41	0.05	-4.66	10.02	1.35
Dieting	0.23	-6.35	7.03	1.31	0.18	-5.93	5.23	1.19
Low Meat	-0.14	-3.96	10.01	1.29	-0.13	-7.68	6.15	1.16
	Active	*Inactive*			*Active*	*Inactive*		
Physical Activity	527	319			1789	978		
	Current	*Social*	*Never*		*Current*	*Social*	*Never*	
Alcohol	141	90	38		452	219	75	
	Current	*Former*	*Never*		*Current*	*Former*	*Never*	
Smoking	64	70	143		107	226	422	
HRT	79	49	151		133	87	532	

6.3.3 Confounding Factors

As with the analysis in Chapter 5, the models of adjustment within this cross-sectional analysis are:

- Model 1. Adjustment for energy intake.
- Model 2. Adjustment for energy intake, age, BMI, smoking, alcohol consumption, physical activity and HRT.

Gender was not adjusted for in the TWINS due to it being an all-female subset of data from the cohort.

6.3.4 Statistical Analyses

The TWINS-UK data was analysed, as in Chapter 5, using a multi-level model in order to take into account the twin pairs. A mixed effects logistic regression was used, with the models of adjustment outlined, to investigate cross-sectional associations between the independent variables (dietary exposures) and the dependent variable (joint pain and CWP). The statistical software programme Stata, version 12, was used for analysis and $p<0.05$ indicated statistically significant findings.

6.4 TWINS-UK Dietary Pattern and Musculoskeletal Pain Results

TABLE 23. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND KNEE PAIN IN TWINS-UK.

Knee Pain	**Model 1 (n=1133)**		**Model 2 (n=778)**	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	1.00	(0.93, 1.07)	1.02	(0.93, 1.11)
High Alcohol	0.88	(0.80, 0.98)*	0.89	(0.76, 1.04)
Traditional English	1.09	(0.99, 1.20)	1.08	(0.95, 1.22)
Dieting	0.97	(0.86, 1.09)	0.94	(0.81, 1.10)
Low Meat	0.95	(0.83, 1.08)	0.92	(0.77, 1.11)

*p<0.05
**p<0.01

TABLE 24. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND HAND PAIN IN TWINS-UK.

Hand Pain	**Model 1 (n=1135)**		**Model 2 (n=779)**	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	1.04	(0.95, 1.13)	0.99	(0.89, 1.12)
High Alcohol	1.00	(0.89, 1.13)	1.09	(0.90, 1.32)
Traditional English	1.11	(0.98, 1.24)	1.17	(1.00,1.37)

Dieting	0.96	(0.84, 1.11)	1.00	(0.82, 1.21)
Low Meat	1.04	(0.89, 1.22)	0.99	(0.79, 1.24)

*p<0.05
**p<0.01

TABLE 25. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND CWP IN TWINS-UK.

Chronic Widespread Pain	**Model 1 (n=1019)**		**Model 2 (n=941)**	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	0.97	(0.85, 1.10)	1.00	(0.88, 1.14)
High Alcohol	0.95	(0.79, 1.14)	1.03	(0.83, 1.28)
Traditional English	1.26	(1.06, 1.49)**	1.18	(0.99, 1.41)
Dieting	0.99	(0.80, 1.22)	1.13	(0.91, 1.41)
Low Meat	1.21	(0.96, 1.53)	1.21	(0.94, 1.56)

*p<0.05
**p<0.01

Please see the appendix for the results from the analysis between dietary patterns and back, neck and shoulder, elbow and forearm and foot pain analysis.

Analysis of dietary patterns revealed a significant association (p=0.017) between the 'High Alcohol' dietary pattern and knee pain (Table 23), which disappears after adjustment of confounding factors.

Although no statistically significant associations were found between hand pain and the five PCA derived dietary patterns (Table 24), worth noting is the association seen with the 'Traditional English' dietary pattern. This shows a positive association with hand pain which only just lost statistical significance (p=0.051), after adjustment of confounding factors. The dietary pattern 'Traditional English' also shows a significant (p=0.009) positive association with CWP, in model one, but does not remain statistically significant when adjusted for confounding factors (Table 25).

6.5 Discussion

The results from this chapter showed that the 'Traditional English' dietary pattern (or so called 'fatty' diet) increases the risk of hand pain by 17%, which was on the edge of statistical significance (p=0.051), after adjustment for all confounders. However, significance could have been lost due to other factors which are discussed further in Chapter 11. Hand pain, which is closely linked to radiographic changes in the hand, shows the same positive association (when investigating this particular dietary pattern) as the results investigating hand OA in the previous chapter. This dietary pattern also increased the risk of CWP by 26% (p=0.009) (Table 25) and of neck and shoulder pain by 16% (p=0.010) when unadjusted for confounders (see Table 48 in Appendix). It also suggested

an increase in the risk of knee pain (Table 23), elbow and forearm pain and foot pain (see Tables 49 and 50 in Appendix), although these were not statistically significant.

A westernised diet such as the 'Traditional English' (as discussed in Chapter 5) contains high amounts of processed foods and unhealthy snacks, which results in a higher intake of nutrients found to have negative effects on health. The finding that a 'Traditional English' dietary pattern increases the risk of CWP is consistent with results from the 1958 British Birth Cohort Study, which demonstrated that women (aged 45 years) with CWP reported a diet of high fats and low fruit and vegetable consumption twelve years previously, compared to women of the same age who were free of pain (VanDenKerkhof, Macdonald et al. 2011).

The effects and mechanisms of the saturated fat palmitate and ω-6 poly-unsaturated fatty acids have already been discussed in the previous chapter, overviewing the effects on inflammatory mechanisms of action which have been found to play a role in the development of OA. High levels of ω-6 PUFAs are known to be associated not only with inflammation but with chronic pain also (Tokuyama and Nakamoto 2011). Mechanisms by which this may occur are through dihomo-γ-linolenic and docosatetraenoic acid (which, as can be seen in Figure 12, are part of the metabolism of ω-6 PUFAs). Levels of these two fatty acids along with elevated levels of trans-fatty acids have been found to be significantly increased in patients with complex regional pain syndrome (Ramsden, Gagnon et al. 2010).

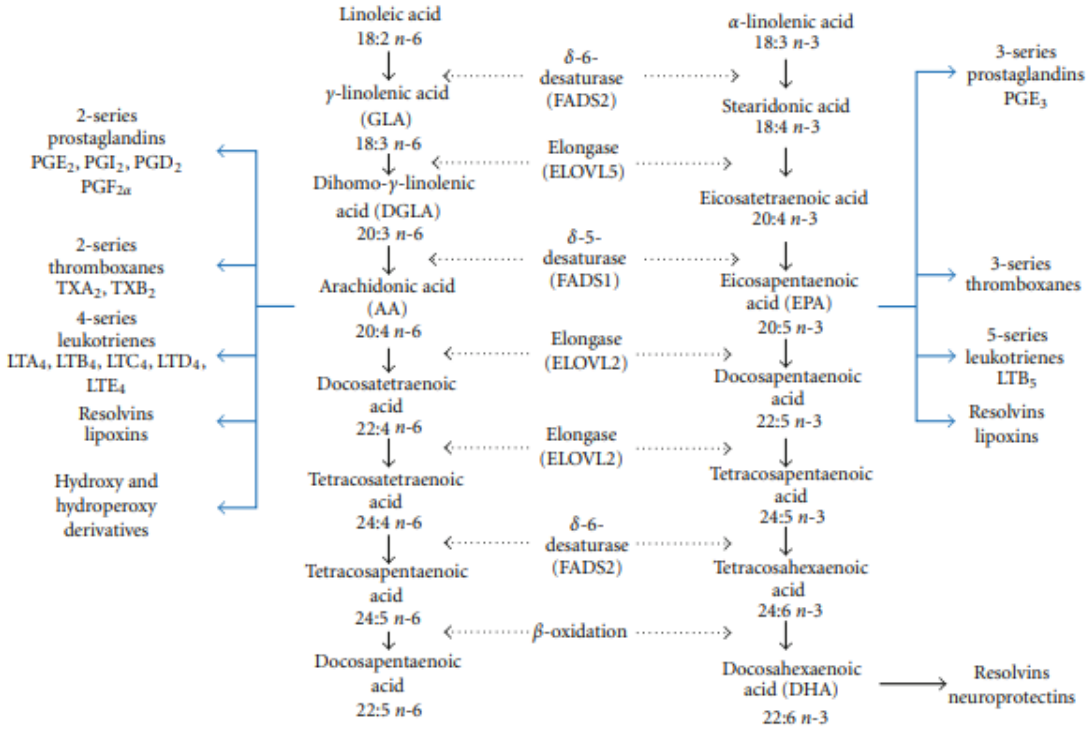

FIGURE 12. COMPLEX METABOLISM OF PUFAS INVOLVING ENZYMES OF DESATURATION, ELONGATION AND B-OXIDATION (PATTERSON, WALL ET AL. 2012).

Another mechanism in which pain may be induced through consuming ω-6 PUFAs (as part of a western diet), is through oxidised linoleic acid metabolites, which can form and activate signalling through transient receptor potential vanilloid (TRPV) (Patwardhan,

Akopian et al. 2010). TRVP1 was the first member of a subgroup of TRP family of ligand-gated ion channels to be discovered and is expressed in a substantial proportion of nociceptors. Activation of TRPV1 occurs through endogenous lipids (e.g. lipoxygenase and phospholipase D metabolites of arachidonic acid) (Cortright and Szallasi 2004). Linoleic acid metabolites are synthesized following stimuli such as peripheral inflammation and have been investigated as a family of endogenous TRPV1 ligands that are able to regulate central sensitization (Patwardhan, Scotland et al. 2009).

Not only are there mechanisms through which ω-6 PUFAs (in high amounts in a western diet) could be having a negative effect on pain there is also a lack of ω-3 PUFAs in a western diet which could also be having a negative effect. ω-3 PUFAs are known to be anti-inflammatory and suppress pain through inhibition of the MAP-Kinase signalling cascade which is involved in the regulation of central sensitization (Woolf and Salter 2000). Alongside inhibition of MAP-K, cytokine activation occurs due to the activation of satellite glial cells (now thought to be a key mechanism of inflammatory pain) (Zhang, Song et al. 2019). Intake of the ω-3 PUFA (α-linoleic acid) has been found to inhibit the production of lysophosphatidic acid, which is strongly related to the development of neuropathic pain (Miyazawa, Ikemoto et al. 2003). DHA also has a calming effect on neuropathic pain and has been reported to have anti-nociceptive effects in a dose dependent manner (Nakamoto, Nishinaka et al. 2010). This effect could be occurring through different mechanisms. Firstly DHA can inhibit the arachidonic acid cascade (Gaudette and Holub 1990). Secondly it can inhibit both calcium channels and voltage gated sodium channels (Xiao, Kang et al. 1995) and influences TRPV1 (Grant, Cottrell et al. 2007).

With the dietary pattern analysis another interesting observation is that consumption of the 'Low Meat' dietary pattern was suggested to decrease the risk of knee pain (Table 23). Although this was not statistically significant, this result, just like those seen in the previous chapter suggest that a diet low in meat, such as the Mediterranean diet, could be beneficial and supports the need for further research as there is limited research investigating the effects of a Mediterranean dietary pattern specifically on OA.

7 Exploring the Mediterranean Dietary Pattern and Radiographic OA, Musculoskeletal Pain and CWP. A Cross-Sectional Analysis using Data from TWINS-UK.

7.1 Chapter Overview

The literature surrounding chronic disease suggests that a Mediterranean style dietary pattern is protective of inflammation, but further investigation is needed into the effects of this dietary pattern on OA. Within TWINS-UK there is a substantial volume of dietary data available for analysis. This allowed for the creation of a Mediterranean style dietary pattern, providing the data for a cross-sectional analysis, investigating associations between radiographic OA variables and pain. The main finding from this study showed that with increased consumption of a Mediterranean dietary pattern, there is a decreased risk in OA of the hand suggesting that consumption of a Med diet could be protective of hand OA.

7.2 Introduction

The Mediterranean diet, as discussed in Chapter 1, emphasizes eating a variety of fruits, vegetables, legumes, nuts, fish, healthy fats (such as olive oil) and limiting the consumption of processed meats whilst consuming alcohol (usually red wine), in moderation and at meal times. This dietary pattern, high in anti-inflammatory compounds and with antioxidant properties, has been the focus of research. This is due to its ability to confer health benefits and has been shown to be protective of a number of chronic diseases. There are a vast number of studies focusing their research efforts on investigating a Mediterranean style dietary pattern, however, research into OA is in single figures and has focused primarily on knee OA. The current evidence for Med diet and OA is not only limited to only a few studies but these are also of cross-sectional design. At present only one study has investigated Med diet and OA longitudinally. Despite hand OA being most prevalent, studies have investigated the effects of Med diet on knee OA, leaving other joint sites unexplored. There is therefore very little evidence for the effects of Med diet on other joints, that are also at risk of OA. This research is the first to address this and investigate the associations that exist between a Med diet and both weight bearing and non-weight bearing joints, along with joint pain and CWP, in an all-female cohort.

7.3 Methods

7.3.1 Mediterranean Dietary Pattern Score

A Mediterranean diet score was created, within TWINS-UK, from the following dietary food groups. Some of the food groups had to be amalgamated to reflect the food groups used in previous publications that best described the Mediterranean diet. Scores were assigned based on the median of the variable of interest as this is a commonly used method throughout the literature (Tong, Wareham et al. 2016). The food groups generated or used

in the creation of the Mediterranean diet pattern score (which was constructed using Microsoft Excel) were: legumes, nuts, eggs, poultry, vegetable (made up of allium, cruciferous, green leafy, other and yellow vegetables), fruit (made up of berries, citrus fruit and other fruit), dairy (made up of both high fat dairy products and low fat dairy products), fish (made up of oily fish, other fish and seafood), cereals (made up of crispbread, high fibre cereals, low fibre cereals, porridge, white and brown bread refined grains and wholemeal bread and grains) and alcohol (made up of wine, beer and spirits and liquor). These food groups are all either high consumption or moderate consumption and were all found to be high within a Mediterranean diet. They were, therefore, assigned a 1 if consumption was high (above the median) and were assigned a 0 if consumption was low (below the median). In contrast, processed meats, meats, cooked potatoes, sweets (made up of high sugar soda, low sugar soda, sweet baked and sweets and condiments) and fats (made up of butter, low fat spread, margarine, poly-unsaturated margarine, high fat dressing and low fat dressing) were all considered food groups found in a non-Mediterranean diet. These were assigned a 1 if consumption was low (below the median) and were assigned a 0 if consumption was high (above the median) providing a Mediterranean dietary pattern score based on the traditional Mediterranean diet constructed by Trichopoulou *et. al.,* 1995 for further analysis.

The following tables show the energy adjusted intakes for those food groups used in the creation of the Mediterranean dietary pattern for cases and non-cases. Across outcome variables the highest intakes are seen for fruits, vegetables, cereals, sweets and fats. For many of the outcome variables intakes for fruit and vegetable consumption are higher in cases compared to non-cases (discussed further in Chapter 11). Please see Tables 26-28 for all TWINS-UK OA variables and Tables 29-35 for all TWINS-UK musculoskeletal pain variables.

TABLE 26. ENERGY ADJUSTED INTAKES OF FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR KNEE OA.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.31	-1.50	25.27	3.17	4.05	-1.52	25.52	2.58
Nuts	0.76	-1.15	15.90	1.97	1.16	-1.18	38.49	2.28
Eggs	1.49	-0.95	7.67	1.36	1.55	-0.84	12.19	1.36
Poultry	1.73	-0.35	6.77	1.17	1.77	-0.42	5.59	1.10
Vegetables	28.55	0.54	219.32	20.68	27.01	-1.42	113.06	12.50
Fruit	18.14	-1.91	68.65	11.45	17.33	-5.47	92.14	11.11
Dairy	12.61	0.62	42.37	5.96	12.33	-1.73	55.34	6.16
Fish	2.17	-0.45	9.26	1.74	2.13	-0.70	15.31	1.66
Cereals	21.89	-10.14	65.10	10.57	21.60	-1.11	63.85	9.42
Alcohol	4.11	-2.11	25.41	5.69	5.08	-2.80	63.89	6.99
Processed Meats	2.75	-1.02	10.28	1.86	2.63	-1.31	21.03	2.06
Meats	2.49	-1.50	9.87	1.85	2.47	-1.32	10.77	1.73
Cooked Potatoes	4.41	-0.54	40.03	4.06	3.73	-1.00	40.83	2.28
Sweets	23.13	-7.35	85.51	16.70	23.86	-8.16	114.65	16.91
Fats	15.14	-4.69	197.05	18.92	14.18	-8.15	89.08	9.50

TABLE 27. ENERGY ADJUSTED INTAKES FOR FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR HIP OA.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.04	-0.49	13.68	2.67	4.07	-0.85	25.52	2.65
Nuts	1.07	-0.73	21.97	2.72	1.19	-1.18	38.49	2.27
Eggs	1.62	-0.33	7.12	1.45	1.50	-0.84	10.20	1.32

Poultry	1.46	-0.26	4.62	1.04	1.81	-0.37	11.51	1.17
Vegetables	25.30	7.05	67.64	11.45	27.70	-1.42	219.32	14.27
Fruit	16.87	-0.81	49.68	10.43	17.51	-5.47	92.14	11.43
Dairy	12.60	3.65	32.80	5.85	12.28	-1.73	50.01	5.93
Fish	1.95	-0.26	6.32	1.52	2.18	-0.70	15.31	1.68
Cereals	19.66	-0.67	40.45	9.95	21.56	-10.14	65.10	9.57
Alcohol	3.84	-1.34	50.92	7.25	5.11	-2.80	63.89	6.45
Processed Meats	2.71	-0.74	18.85	2.60	2.66	-1.31	15.12	1.96
Meats	2.35	-0.69	8.88	1.87	2.45	-1.32	10.77	1.74
Cooked Potatoes	4.37	-0.43	31.70	3.60	3.74	-0.88	17.85	2.19
Sweets	23.26	-10.13	68.11	16.16	23.31	-7.54	98.88	16.51
Fats	13.34	-1.91	68.79	11.67	14.53	-8.15	197.05	11.64

TABLE 28. ENERGY ADJUSTED INTAKES FOR FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR HAND OA.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.11	-0.14	16.10	2.69	4.15	-1.52	25.52	2.66
Nuts	0.77	-0.96	17.50	1.73	1.15	-1.18	38.49	2.23
Eggs	1.48	-0.53	6.28	1.20	1.55	-0.95	12.19	1.36
Poultry	1.67	-0.28	5.69	1.09	1.78	-0.42	11.51	1.15
Vegetables	27.48	4.55	98.14	13.15	23.86	-1.42	219.32	15.81
Fruit	18.42	-0.73	88.91	12.65	15.03	-5.47	92.14	11.64
Dairy	12.65	1.84	53.18	6.42	10.78	-1.73	55.34	7.05
Fish	2.06	-0.37	8.79	1.55	1.87	-0.70	18.87	1.77
Cereals	21.33	-1.59	71.90	10.25	18.66	-10.14	63.85	11.42

Alcohol	4.19	-1.85	60.04	6.54	4.35	-2.80	63.89	6.47
Processed Meats	2.65	-0.63	9.80	1.79	2.65	-1.31	18.85	2.00
Meats	2.72	-0.56	10.45	1.85	2.47	-1.50	10.77	1.74
Cooked Potatoes	4.45	-0.73	31.70	2.98	3.76	-1.00	40.83	2.46
Sweets	24.08	-7.33	114.65	16.61	20.28	-10.13	105.67	17.31
Fats	14.89	-5.74	153.13	13.45	12.23	-8.15	197.05	10.68

TABLE 29. ENERGY ADJUSTED INTAKES FOR FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR KNEE PAIN.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.36	-1.56	31.77	2.91	4.03	-1.60	18.02	2.65
Nuts	1.20	-1.12	38.49	2.26	1.43	-1.33	40.71	2.45
Eggs	1.48	-1.00	17.53	1.37	1.44	-0.64	18.15	1.43
Poultry	1.94	-0.51	7.26	1.21	1.90	-0.54	11.51	1.21
Vegetables	28.69	-2.58	233.92	14.77	28.07	-3.89	219.32	14.43
Fruit	18.09	-7.63	92.14	11.45	17.17	-8.78	92.61	11.33
Dairy	12.21	-1.81	53.18	6.32	11.67	-4.86	57.48	5.87
Fish	2.29	-0.84	18.87	1.88	2.15	-0.92	13.43	1.68
Cereals	21.18	-6.40	96.64	9.73	21.03	-9.09	82.40	9.28
Alcohol	4.97	-2.61	52.05	6.67	5.94	-2.87	84.46	7.69
Processed Meats	2.72	-1.31	18.85	2.07	2.67	-1.58	15.12	1.97
Meats	2.49	-1.32	10.67	1.78	2.26	-1.46	9.87	1.68
Cooked Potatoes	3.66	-0.95	24.00	2.11	3.48	-1.69	18.30	2.00
Sweets	22.94	-18.81	107.43	16.46	22.81	-16.12	123.53	16.64
Fats	12.76	-6.66	99.87	8.83	13.33	-7.56	114.16	8.79

TABLE 30. ENERGY ADJUSTED INTAKES FOR FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR HAND PAIN.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.33	-1.60	20.36	2.66	4.08	-1.56	31.77	2.78
Nuts	1.31	-1.10	24.68	2.19	1.38	-1.33	40.71	2.48
Eggs	1.49	-1.00	17.54	1.48	1.44	-0.69	18.15	1.38
Poultry	1.93	-0.51	6.99	1.20	1.92	-0.54	11.51	1.22
Vegetables	29.26	-3.07	113.57	14.13	27.95	-3.89	233.92	14.80
Fruit	18.24	-7.63	92.14	11.50	17.23	-8.78	92.61	11.48
Dairy	12.03	-4.86	51.34	5.90	11.77	-3.51	57.48	6.14
Fish	2.31	-0.84	18.87	1.85	2.16	-0.92	18.33	1.75
Cereals	20.83	-6.40	76.01	9.05	21.05	-9.09	96.64	9.66
Alcohol	5.17	-2.61	84.46	7.11	5.73	-2.87	81.56	7.41
Processed Meats	2.73	-1.31	15.12	2.01	2.68	-1.58	18.85	2.00
Meats	2.52	-1.32	10.67	1.81	2.28	-1.46	10.45	1.68
Cooked Potatoes	3.67	-1.07	24.00	2.07	3.50	-1.69	18.30	2.04
Sweets	22.84	-16.12	123.53	16.49	22.78	-18.81	118.50	16.66
Fats	12.73	-6.66	72.44	8.45	13.14	-7.56	114.16	9.02

TABLE 31. ENERGY ADJUSTED INTAKES FOR FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR NECK AND SHOULDER PAIN.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.27	-1.03	31.77	2.82	4.04	-1.60	20.36	2.68
Nuts	1.40	-1.33	40.71	2.50	1.31	-1.16	38.49	2.30
Eggs	1.49	-1.00	17.94	1.45	1.43	-0.64	18.15	1.36

Poultry	1.93	-0.53	11.51	1.25	1.89	-0.54	7.15	1.17
Vegetables	28.81	-3.89	233.92	15.41	27.78	-2.59	113.57	13.76
Fruit	17.58	-7.63	92.14	11.41	17.56	-8.78	92.61	11.52
Dairy	11.88	-1.81	57.48	6.28	11.87	-4.86	47.74	5.74
Fish	2.17	-0.92	18.33	1.71	2.23	-0.76	18.87	1.83
Cereals	20.95	-6.40	76.01	9.64	21.15	-9.09	96.64	9.30
Alcohol	5.40	-2.87	81.56	7.06	5.67	-2.80	84.46	7.45
Processed Meats	2.77	-1.31	15.12	2.07	2.64	-1.58	21.03	2.00
Meats	2.44	-1.32	10.67	1.81	2.27	-1.46	10.45	1.65
Cooked Potatoes	3.64	-1.34	24.00	2.09	3.45	-1.69	17.76	2.00
Sweets	23.16	-18.81	123.53	16.66	22.53	-11.19	118.50	16.33
Fats	13.04	-7.56	99.87	8.82	13.12	-6.79	114.16	8.86

TABLE 32. ENERGY ADJUSTED INTAKES FOR FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR ELBOW AND FOREARM PAIN.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.35	-0.87	14.52	2.68	4.12	-1.60	31.77	2.77
Nuts	1.32	-1.10	40.71	2.86	1.37	-1.33	24.68	2.29
Eggs	1.51	-1.00	17.53	1.51	1.45	-0.69	18.15	1.38
Poultry	2.01	-0.44	7.20	1.25	1.89	-0.54	11.51	1.19
Vegetables	28.21	-2.59	98.07	13.54	28.33	-3.89	233.92	14.84
Fruit	18.06	-4.24	81.14	11.65	17.47	-8.78	92.61	11.36
Dairy	11.93	-4.86	50.01	5.96	11.94	-3.51	57.48	6.11
Fish	2.26	-0.84	11.37	1.73	2.20	-0.92	18.87	1.79
Cereals	20.75	-4.29	59.21	9.01	21.15	-9.09	96.64	9.51

Alcohol	5.43	-2.09	84.46	7.75	5.58	-2.87	81.56	7.18
Processed Meats	2.81	-1.31	12.48	2.07	2.67	-1.58	18.85	1.99
Meats	2.48	-1.22	10.67	1.85	2.32	-1.46	10.45	1.68
Cooked Potatoes	3.59	-0.95	18.30	2.06	3.54	-1.69	24.00	2.04
Sweets	23.80	-16.12	123.53	17.87	22.58	-11.19	118.50	16.16
Fats	12.95	-7.56	63.48	8.56	13.06	-6.79	114.16	8.82

TABLE 33. ENERGY ADJUSTED INTAKES FOR FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR FOOT PAIN.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.10	-1.60	42.03	2.83	4.07	-1.60	31.77	2.75
Nuts	1.34	-1.33	38.49	2.43	1.37	-1.33	40.71	2.45
Eggs	1.46	-1.11	17.94	1.40	1.42	-0.84	18.15	1.38
Poultry	1.92	-0.62	11.51	1.24	1.90	-0.54	11.51	1.20
Vegetables	25.99	-10.42	233.92	15.76	28.26	-3.89	233.92	14.88
Fruit	16.08	-8.78	92.61	12.06	17.37	-7.63	92.61	11.51
Dairy	10.96	-5.79	57.48	6.75	11.90	-3.51	52.79	6.08
Fish	2.01	-0.93	21.64	1.83	2.18	-0.92	18.33	1.75
Cereals	19.37	-9.09	96.64	10.82	20.95	-3.64	96.64	9.44
Alcohol	5.13	-2.87	84.46	7.27	5.67	-2.87	84.46	7.31
Processed Meats	2.74	-1.82	22.10	2.07	2.68	-1.58	18.85	2.01
Meats	2.35	-1.69	10.77	1.74	2.30	-1.46	10.45	1.67
Cooked Potatoes	3.58	-1.69	40.86	2.37	3.49	-1.34	24.00	2.02
Sweets	21.43	-18.81	130.61	17.44	22.63	-18.81	118.50	16.40
Fats	12.21	-9.99	153.13	9.48	12.73	-7.56	99.87	8.35

TABLE 34. ENERGY ADJUSTED INTAKES OF FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR BACK PAIN.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.00	-0.64	14.13	2.45	3.96	-0.68	25.27	2.59
Nuts	1.38	-1.12	36.58	2.64	1.08	-1.33	38.49	2.04
Eggs	1.44	-1.05	6.07	1.18	1.44	-0.84	12.02	1.27
Poultry	1.95	-0.44	7.15	1.19	1.86	-0.51	5.87	1.14
Vegetables	28.46	1.15	104.25	13.55	27.04	-3.07	233.92	13.47
Fruit	17.74	-7.63	63.74	10.35	16.77	-5.39	92.61	11.08
Dairy	12.24	-5.79	42.76	6.00	12.02	-4.86	55.34	5.68
Fish	2.14	-0.64	11.34	1.63	2.06	-0.92	18.87	1.61
Cereals	22.20	-1.11	83.05	10.39	21.43	-10.14	71.90	9.30
Alcohol	5.33	-2.61	59.97	7.42	5.49	-2.80	81.56	7.12
Processed Meats	2.55	-1.29	10.19	1.78	2.62	-1.31	18.35	1.93
Meats	2.23	-1.20	9.86	1.66	2.35	-1.32	10.77	1.69
Cooked Potatoes	3.65	-0.62	40.86	2.69	3.58	-0.88	40.83	2.19
Sweets	24.96	-14.41	114.65	18.07	23.61	-9.91	118.05	16.29
Fats	13.49	-9.99	99.87	9.89	13.81	-8.15	197.05	10.25

TABLE 35. ENERGY ADJUSTED INTAKES FOR FOOD GROUPS USED IN MEDITERRANEAN DIETARY PATTERN FOR CWP.

	OA Cases				**Non cases**			
Food Groups	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Legumes	4.44	-1.52	19.65	2.79	4.00	-1.60	31.77	2.69
Nuts	1.28	-1.12	36.12	2.68	1.50	-1.33	40.71	2.57
Eggs	1.49	-1.00	9.51	1.36	1.43	-0.95	18.21	1.48

Poultry	1.99	-0.42	7.26	1.31	1.89	-0.62	11.51	1.20
Vegetables	29.43	-3.07	111.77	14.18	28.08	-3.89	233.92	14.62
Fruit	18.28	-4.24	92.14	11.57	17.56	-8.78	92.61	11.59
Dairy	12.07	-1.81	51.34	6.33	11.84	-4.86	47.74	5.97
Fish	2.31	-0.84	18.33	1.94	2.18	-0.92	18.87	1.72
Cereals	20.53	-6.40	64.28	9.27	20.86	-9.09	96.64	9.19
Alcohol	4.99	-1.88	52.05	6.98	5.84	-2.87	81.56	7.34
Processed Meats	2.84	-1.23	18.85	2.19	2.69	-1.82	22.10	2.05
Meats	2.50	-1.32	10.67	1.91	2.28	-1.69	10.77	1.66
Cooked Potatoes	3.71	-1.00	16.96	1.95	3.49	-1.69	40.03	2.29
Sweets	23.53	-18.81	123.53	16.76	22.54	-16.12	118.50	16.19
Fats	12.81	-6.66	72.13	8.55	12.85	-7.56	114.16	8.46

7.3.2 Dependent Variables

The same radiographic OA variables (hip, knee and hand OA) and the pain variables (knee, hand, back, neck and shoulder, elbow and forearm, foot pain and CWP), analysed in Chapters 5 and 6, are the dependent variables of interest for this analysis. Please see Chapters 5.3.and 6.3 for outcome assessment criteria and characteristics tables.

7.3.3 Confounding Factors

As with the analysis in Chapters 5 and 6, the models used adjust for total energy intake and for the most commonly known confounders; age, BMI, physical activity, smoking history, alcohol consumption and the use of Hormone Replacement Therapy which have all been suggested to have relevance in the development of chronic OA. The models of adjustment are:

- Model 1. Adjustment for energy intake.
- Model 2. Adjustment for energy intake, age, BMI, smoking, alcohol consumption, physical activity and HRT.

Gender was not adjusted for in the TWINS-UK analysis, due to it being an all-female subset of data from the cohort.

7.3.4 Statistical Analysis

The TWINS-UK data was analysed, as in Chapters 5 and 6, using a multi-level model in order to take into account the twin pairs. A mixed effects logistic regression was used, with the models of adjustment above, to investigate cross-sectional associations between the independent variable (Mediterranean dietary pattern) and the dependent variables (Radiographic OA, joint pain and CWP). The statistical software programme Stata, version 12, was used for analysis and $p<0.05$ indicated statistically significant findings.

7.4 TWINS-UK Mediterranean Diet Results

Table 36 shows associations between the Mediterranean dietary pattern and the dependent variables of interest.

TABLE 36. OR AND 95% CI FOR ASSOCIATIONS BETWEEN THE MEDITERRANEAN DIETARY PATTERN AND THE OA/PAIN VARIABLES OF INTEREST IN TWINS-UK.

Mediterranean Dietary Pattern	**Model 1**		**Model 2**	
Variable	**OR**	**95% CI**	**OR**	**95% CI**
Knee OA (model 1 n=1417, model 2 n=800)	0.96	(0.90, 1.03)	1.03	(0.94, 1.12)
Hip OA (model 1 n=1102, model 2 n=626)	0.89	(0.80, 1.00)*	0.95	(0.82, 1.10)
Hand OA (model 1 n=1592, model 2 n=901)	0.90	(0.83, 0.98)*	0.88	(0.79, 0.99)*
Knee Pain (model 1 n=1133, model 2 n=778)	1.00	(0.95, 1.06)	1.01	(0.94, 1.08)
Hand Pain (model 1 n=1135, model 2 n=779)	1.01	(0.95, 1.08)	1.01	(0.92, 1.10)
Foot Pain (model 1 n=1090, model 2 n=746)	1.01	(0.95, 1.08)	1.02	(0.93, 1.12)
Neck and Shoulder Pain (model 1 n=1166, model 2 n=806)	0.99	(0.93, 1.05)	1.06	(0.98, 1.14)
Elbow and Forearm Pain (model 1 n=1113, model 2 n=770)	0.98	(0.91, 1.05)	0.94	(0.85, 1.03)
Back pain (model 1 n=1012, model 2 n=597)	0.93	(0.83, 1.04)	0.88	(0.76, 1.02)
CWP (model 1 n=1019, model 2 n=941)	0.97	(0.88, 1.06)	1.01	(0.91, 1.11)

*p<0.05
**p<0.01

Table 36 shows an inverse association between the Mediterranean dietary pattern and hip OA (p=0.049) although this does not remain statistically significant with adjustment of confounding factors. However, there is a consistent statistically significant inverse association (model 1 p=0.015 and model 2 p=0.027) seen between the Mediterranean dietary pattern and hand OA.

7.5 Discussion

The Mediterranean diet (a dietary pattern which is rich in complex carbohydrates and fibres and low in animal proteins and fats) is shown in this research to decrease the risk of hip OA (p=0.049) although this lost statistical significance after further adjustment of confounding factors). However, the consumption of a Mediterranean style diet showed an inverse association with hand OA (p=0.027) in both model one and model two and there are multiple ways in which the mediterranean diet could be demonstrating this protective effect on Osteoarthritis.

A way in which the Med diet may be having a protective effect on hand OA, therefore, is through the effect of the fatty acid content of foods, found within a Mediterranean style diet, on inflammation.

When ω-3 PUFAs are metabolised, there is a shift towards an anti-inflammatory profile (as discussed in Chapter 1). ω-3 fatty acids play an important role in chondrocytes, inhibiting mTOR and activating autophagy as a protective mechanism for cartilage maintenance. Fatty acids are essential and the effects that they have on chondrocyte metabolism support potential benefits of having ω-3 PUFA content within the diet (Villalvilla, Gómez et al. 2013).

Previous research has found that ω-3 PUFAs can significantly enhance wound repair (Villalvilla, Gómez et al. 2013). Krill oil supplements have become popular as an additional source of ω-3 FAs (Tou, Jaczynski et al. 2007). A randomised double blind trial found significant reductions in pain, stiffness and CRP, when 300mg of krill oil was consumed in those with OA confirmed at the hip and knee (Deutsch 2007). The MOST study looked at individuals either suffering from or at high risk of knee OA and the relationship with dietary fatty acids to help establish dietary recommendations for fatty acid intake in order to reduce inflammation in OA. High levels of total ω-3 PUFAs had lower patellofemoral cartilage loss. Higher synovitis severity was seen with high levels of ω-6 PUFAs concluding that diet is, therefore, a focus to influence and help maintain the structural integrity of the knee (Baker, Matthan et al. 2012).

There have been many more studies looking into the different characteristics of OA in relation to dietary fatty acids (Lajeunesse and Reboul 2007), which support the findings found by the MOST study. In osteoarthritic bone, for example, levels of ω-6 PUFAs were found to be higher (Plumb and Aspden 2004), whereas the regulation of catabolic factors by ω-3 PUFAs in turn regulates cartilage destruction (Curtis, Rees et al. 2002). Arachidonic acid, as mentioned in Chapter 1, is an omega-6 PUFA, which can modulate inflammation. It is metabolised by phospholipase C into eicosanoids, of which 2-series prostaglandins and 4-series leukotrienes have pro-inflammatory effects when in excess (Ferretti, Nelson et al. 1997). However, omega-3 PUFAs can reduce the effect of arachidonic acid and the formation of eicosanoids by impacting on the composition of plasma phospholipid fatty acids and alternatively producing 3-series and 5- series prostaglandins and leukotrienes (Li, Birdwell et al. 1994).

With the results from TWINS-UK analysis in this chapter being focused around a very different dietary pattern to the one studied in the previous two chapters, it is important to think about the differences between a 'Traditional English' dietary pattern and a Mediterranean dietary pattern and in which foods the nutrients are found. Figure 13 shows the foods in which ω-3 and ω-6 PUFAs are found and their abundance within these foods.

Fat type	LA	ALA	AA	EPA + DHA
Saturated				
Lard	8600	1000	1070	
Butter fat	2300	1400		
Coconut oil	1400			
Beef tallow	80			
Unsaturated				
(1) Monounsaturated				
Peanut oil	23900			
Pecans	20600	1000		
Almonds	9860	260		
Olive oil	8000	950		
Avocado	1970			
(2) Polyunsaturated				
Omega-6				
Safflower oil	74000	470		
Sunflower oil	60200	500		
Soybean oil	53400	7600		
Corn oil	50000	900		
Cotton seed oil	47800	1000		
Walnut	34100	6800	590	
Brazil nut	24900			
Omega-3				
Linseed oil	13400	55300		
Canola oil	19100	8600		
Salmon	440	550	300	1200
Tuna	260	270	280	400
Herring	150	62	37	1700
Trout	74		30	500
Cod	4	2	3	300

FIGURE 13. FATTY ACID CONTENT OF FOOD SOURCES OF DIETARY FATS IN MG/100G EDIBLE FOOD (PATTERSON, WALL ET AL. 2012).

Unlike the western diet which is also rich in fats, the majority of the fat content in the Med diet (approximately 85%) is due to the single component olive oil, making it high in MUFAs and low in saturated fats and cholesterol (Serra-Majem, De La Cruz et al. 2003). The presence of olive oil also facilitates the consumption of other food components, such as vegetables and legumes that promote health (Oliviero, Spinella et al. 2015). One of the ways in which olive oil exerts its beneficial effects is due to the component oleic acid interfering with signal transduction pathways and cytokine production. Due to its ability to modulate expression of pleitrophic genes, oleic acid has been shown, using rat models, to regulate genes within signal transduction pathways. It also decreases the production of pro-inflammatory cytokines IL-1β and IL-6 (Magdalon, Vinolo et al. 2012). Lubricin expression in cartilage has also been restored after supplementation with extra virgin olive oil (containing high amounts of the ω-9 FA oleic acid) (Magdalon, Vinolo et al. 2012). Diets rich in monounsaturated fats have been shown to be effective at lowering cholesterol levels due to the 17.9% reduction in serum LDL cholesterol level. This was noted in those participants who were given a mixed diet rich in MUFAs and a 12.9% reduction was noted in those on the same mixed diet but rich in PUFAs (Mensink and Katan 1989).

The second way in which the Mediterranean diet could be having a beneficial effect, is through the action of polyphenol compounds on inflammation. Oleuropein found in olives,

hydroxytyrosol found in extra virgin olive oil and resveratrol found in red grapes (and red wine) are suggested to be able to block inflammatory pathways. They have been shown to inhibit the activation of the NF-κB and AP-1 transcription factors (Carluccio, Siculella et al. 2003) and influence COX-2, prostanoid and MMP activity (Scoditti, Calabriso et al. 2012). Compounds found within olive oil specifically, are noted to have anti-inflammatory effects concerning joint inflammation. The first being hydroxytyrosol, which has shown significant improvement in disease activity (in rat models) by down-regulating COX-2 and iNOS expression (Silva, Sepodes et al. 2015). The second is secoiridoid oleocanthal, which has been shown to downregulate pro-inflammatory cytokines such as IL-6, IL-1 and TNFα in chondrocytes (Scotece, Gómez et al. 2012). The third is oleuropein aglycone, which has also been shown to regulate the inflammatory response, improve tissue damage and reduce the plasma levels of pro-inflammatory cytokines (Impellizzeri, Esposito et al. 2011).

Polyphenol extract from olive oil has also been shown to reduce joint oedema, bone erosion and cartilage degradation by influencing the JNK, p38, STAT3 and NF-κB signalling pathways (Rosillo, Alcaraz et al. 2014). A more recent study found that polyphenol extract was able to reduce the production of pro-inflammatory mediators in human synovial fibroblasts. As with the animal models mentioned above, this study also suggested that protective effects could be linked to the inhibition of the MAPK and NF-κB signalling pathways (Rosillo, Alarcón-de-la-Lastra et al. 2019).

A recent study investigated the antioxidant hydroxytyrosol, which has been found to protect chondrocytes from DNA damage and even cell death caused by oxidative stress. Findings indicated that chondrocyte autophagy markers were elevated and the accumulation of the deacetylase Sirtuin-1 (SIRT-1), in the nucleus, was needed for hydroxytyrosol to perform this protective effect (Cetrullo, D'Adamo et al. 2016). The Sirtuin (SIRT) family of genes are activated by the consumption of foods that contain the highest levels of polyphenols and have shown hugely beneficial effects in health and disease. Therefore the finding that SIRT-1 is required for protective effects to take place is not surprising as olive oil is considered a SIRT food. One biological mechanism, discussed in the literature, through which the Mediterranean diet may have a protective effect is the Nrf2 signalling pathway (a known antioxidant response mechanism) (Jain, Lamark et al. 2010). It has been noted that hydroxytyrosol increases p62 transcription (Cetrullo, D'Adamo et al. 2016) which is an activator of Nrf2 (Jain, Lamark et al. 2010). The way in which the Mediterranean diet has a protective effect could also be through the inflammatory mediator hs-CRP, which plays a key role in the inflammatory response.

Clinical studies that have taken place in order to investigate the efficacy of a Med diet on joint disease activity have focused their attention on rheumatoid arthritis. These studies have assessed the effects of consumption of a Med diet by comparing health assessment questionnaires and visual analogue scale pain scores as primary variables but with mixed results (Oliviero, Spinella et al. 2015). Skoldstam et al., also measured CRP and cholesterol levels and found significant improvement in these. Also noted was improvement in questionnaires used as primary efficacy variables (in respect to baseline values) in comparison to the control group where no change was found (Sköldstam, Hagfors et al. 2003). There is a need, therefore, to investigate other cohorts to confirm the associations between a Med diet and hand OA, found in this research. Clinical studies should also be conducted in order to see whether there is a true and direct protective effect of consuming a Mediterranean style diet on hand OA.

8 An Investigation into Serum Blood Lipids and Radiographic OA, Musculoskeletal Pain and Chronic Widespread Pain. A Cross-Sectional Analysis using Data from TWINS-UK.

8.1 Chapter Overview

The analysis within the TWINS-UK cohort shows that an increase in the consumption of a 'Traditional English' dietary pattern increases the risk of OA/pain. The cross-sectional case-control analysis within EPIC-Norfolk found that triglycerides are positively associated with pain. With excessive exposure to dietary lipids being one of the causes of inflammatory responses (Pfluger, Herranz et al. 2008) and with dietary fats affecting circulating lipid levels, there is, therefore, a need to further investigate the effect of diet on OA/pain by analysing serum blood lipid data. The main finding is that triglycerides significantly increased the risk of hip OA (p=0.009), hand OA (p=0.003), elbow and forearm pain (p=0.005) and CWP (p=0.020).

8.2 Introduction

Nutrient excess and obesity can lead to a complete lipid overload, alongside increased levels of circulating fatty acids and lipotoxicity. Dietary fatty acids, consumed as part of the diet, (depending on the type and quantity) have an effect on plasma cholesterol which circulates in the blood as part of lipoprotein complexes, such as LDL and HDL (Ertunc and Hotamisligil 2016). It is known that lipids play a role and are important in metabolic and homeostatic processes, such as immune function, energy metabolism, organelle homeostasis, communication between organs and cell survival. Despite their necessity and their playing a key role within the joint, when in excess or when their composition or balance in metabolism is altered, they can lead to metabolic disturbance, organelle dysfunction, cell death and chronic inflammation. This occurs through lipids becoming incorporated in signalling cascades which are damaging to cell and tissue function (Ertunc and Hotamisligil 2016).

The previous chapters have investigated associations, between diet and OA/pain, many of which support the theory that a Western diet is pro-inflammatory and the Mediterranean diet is anti-inflammatory (Landaeta-Diaz, Fernandez et al. 2013). The findings from EPIC-Norfolk showed that nutritional food groups high in fats are positively associated with hip and knee OA. The EPIC-Norfolk analysis also showed that triglycerides are positively associated with knee OA and knee pain. The findings from the TWINS-UK dietary analysis showed that a 'Traditional English' dietary pattern (defined as a dietary pattern high in fats/fatty foods) is positively associated with hand OA and is also positively associated with knee and hand pain (although not significant). With this in mind, this next chapter investigates biological mechanisms by which diet could be having an effect on OA/pain. Serum blood lipid data from TWINS-UK was, therefore, analysed to explore associations between lipids and OA/pain in an attempt to replicate findings seen in EPIC-Norfolk.

8.3 Methods

8.3.1 Serum Blood Lipid Data

Blood lipid concentrations were determined by taking venous blood samples from participants in the morning after an overnight fast. Blood was extracted from twins within each pair less than 5 minutes apart and lipid levels were measured using a Cobas Fara machine. The serum blood lipid data, from TWINS-UK, consists of triglycerides, cholesterol, HDL, LDL, apoA1 and apoB1. Triglycerides, cholesterol and HDL were determined by the colorimetric enzymatic method, Apolipoprotein A1 and B1 by immunoturbidometric method and LDL levels were estimated using the Friedewald equation. These lipids are investigated in this chapter to improve our understanding of how dietary factors can influenc OA/pain.

The following tables show the levels of blood lipids across cases and non-cases for all knee, hip and hand OA variables (Table 37) and for all musculoskeletal pain variables (Table 38). As can be seen from these tables, the mean levels of triglycerides, LDL, Cholesterol and ApoB1 are elevated in OA and pain cases compared to non-cases. Levels of HDL are lower across pain cases compared to non-cases and levels of APOA1 vary across outcome variables when comparing cases and non-cases.

TABLE 37. CHARACTERISTICS OF BLOOD LIPID DATA BY OA SITE (MMOL/L).

	OA Cases				**Non cases**			
Knee OA	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Apolipoprotein A1	1.70	0.89	2.65	0.31	1.71	0.83	2.85	0.32
Apolipoprotein B1	1.31	0.37	2.34	0.37	1.23	0.43	2.33	0.35
Cholesterol	6.19	3.00	9.16	1.28	5.87	2.82	9.28	1.12
High Density Lipoprotein	1.49	0.67	2.51	0.37	1.54	0.60	2.67	0.38
Low Density Lipoprotein	3.97	1.32	6.99	1.14	3.71	0.91	7.32	1.03
Triglycerides	1.42	0.07	3.71	0.68	1.28	0.27	3.62	0.64
Hip OA								
Apolipoprotein A1	1.71	1.16	2.40	0.30	1.72	0.83	2.85	0.32
Apolipoprotein B1	1.39	0.68	2.34	0.38	1.24	0.37	2.33	0.35
Cholesterol	6.14	3.92	8.68	1.20	5.87	2.82	9.28	1.17
High Density Lipoprotein	1.53	0.89	2.60	0.38	1.53	0.60	2.67	0.39
Low Density Lipoprotein	3.81	2.17	6.15	1.05	3.72	0.91	7.32	1.07
Triglycerides	1.57	0.43	3.60	0.82	1.27	0.27	3.62	0.62
Hand OA								
Apolipoprotein A1	1.72	0.83	2.84	0.33	1.71	0.85	2.85	0.32
Apolipoprotein B1	1.39	0.45	2.30	0.35	1.24	0.37	2.34	0.35
Cholesterol	6.41	3.47	9.10	1.11	5.87	2.82	9.28	1.13
High Density Lipoprotein	1.53	0.60	2.58	0.38	1.53	0.60	2.67	0.38
Low Density Lipoprotein	4.12	1.11	6.97	0.99	3.72	0.91	7.32	1.05
Triglycerides	1.51	0.39	3.71	0.71	1.28	0.07	3.60	0.62

TABLE 38. CHARACTERISTICS OF BLOOD LIPID DATA BY MUSCULOSKELETAL PAIN SITE (MMOL/L).

	Pain Cases				**Non cases**			
Knee Pain	*mean*	*min*	*max*	*SD*	*mean*	*min*	*max*	*SD*
Apolipoprotein A1	1.70	0.73	2.84	0.32	1.68	0.82	2.85	0.33
Apolipoprotein B1	1.20	0.32	2.34	0.36	1.11	0.36	2.22	0.34
Cholesterol	5.77	2.50	9.30	1.19	5.53	2.45	9.28	1.19
High Density Lipoprotein	1.53	0.60	2.67	0.37	1.56	0.56	2.72	0.38
Low Density Lipoprotein	3.64	1.11	7.18	1.11	3.44	0.56	7.32	1.06
Triglycerides	1.28	0.07	3.71	0.63	1.14	0.30	3.60	0.57
Hand Pain								
Apolipoprotein A1	1.70	0.73	2.84	0.33	1.68	0.82	2.85	0.32
Apolipoprotein B1	1.19	0.32	2.34	0.37	1.12	0.36	2.26	0.35
Cholesterol	5.81	2.45	9.30	1.24	5.53	2.77	9.28	1.16
High Density Lipoprotein	1.54	0.56	2.63	0.38	1.55	0.60	2.72	0.38
Low Density Lipoprotein	3.66	1.01	6.97	1.12	3.45	0.56	7.32	1.06
Triglycerides	1.28	0.07	3.71	0.64	1.15	0.30	3.60	0.56
Neck and Shoulder Pain								
Apolipoprotein A1	1.68	0.73	2.84	0.33	1.69	0.83	2.85	0.31
Apolipoprotein B1	1.16	0.32	2.34	0.36	1.15	0.37	2.33	0.35
Cholesterol	5.65	2.45	9.30	1.20	5.63	2.50	9.32	1.20
High Density Lipoprotein	1.53	0.56	2.72	0.38	1.57	0.60	2.70	0.37
Low Density Lipoprotein	3.54	0.88	6.99	1.10	3.52	0.56	7.32	1.08
Triglycerides	1.22	0.07	3.62	0.60	1.18	0.30	3.71	0.60
Elbow and Forearm Pain								
Apolipoprotein A1	1.68	0.91	2.84	0.32	1.69	0.73	2.85	0.32
Apolipoprotein B1	1.18	0.49	2.34	0.37	1.14	0.32	2.33	0.35

Cholesterol	5.72	3.09	9.30	1.24	5.59	2.45	9.23	1.18
High Density Lipoprotein	1.50	0.61	2.67	0.37	1.57	0.56	2.72	0.38
Low Density Lipoprotein	3.61	0.88	6.88	1.12	3.49	0.56	7.18	1.07
Triglycerides	1.30	0.30	3.62	0.64	1.16	0.07	3.71	0.58
Foot Pain								
Apolipoprotein A1	1.69	0.72	2.85	0.32	1.69	0.73	2.85	0.33
Apolipoprotein B1	1.16	0.30	2.34	0.35	1.12	0.32	2.33	0.34
Cholesterol	5.66	2.45	9.30	1.18	5.53	2.45	9.30	1.16
High Density Lipoprotein	1.54	0.56	2.72	0.38	1.56	0.56	2.72	0.38
Low Density Lipoprotein	3.55	0.56	7.32	1.08	3.44	0.56	6.97	1.05
Triglycerides	1.20	0.07	3.71	0.60	1.15	0.07	3.71	0.57
Back Pain								
Apolipoprotein A1	1.71	0.98	2.82	0.31	1.69	0.73	2.85	0.35
Apolipoprotein B1	1.17	0.41	2.20	0.34	1.18	0.30	2.33	0.36
Cholesterol	5.74	2.82	9.22	1.23	5.74	2.47	9.28	1.16
High Density Lipoprotein	1.48	0.81	2.70	0.37	1.53	0.60	2.70	0.38
Low Density Lipoprotein	3.65	0.91	6.69	1.10	3.64	0.56	7.32	1.05
Triglycerides	1.28	0.40	3.71	0.65	1.20	0.27	3.60	0.57
CWP								
Apolipoprotein A1	1.70	0.73	2.76	0.33	1.69	0.82	2.85	0.32
Apolipoprotein B1	1.22	0.32	2.34	0.36	1.12	0.33	2.33	0.34
Cholesterol	5.92	2.82	9.30	1.24	5.52	2.45	9.32	1.12
High Density Lipoprotein	1.50	0.66	2.72	0.38	1.57	0.56	2.71	0.38
Low Density Lipoprotein	3.80	0.88	7.18	1.16	3.43	0.56	7.32	1.02
Triglycerides	1.35	0.07	3.62	0.65	1.13	0.30	3.60	0.56

Please see Chapters 5.3.and 6.3 for outcome assessment criteria and characteristics tables for OA and musculoskeletal pain sites.

8.3.2 Statistical Analysis

A mixed effects logistic regression analysis was used to investigate cross-sectional associations between serum blood lipids and the radiographic OA and musculoskeletal pain variables.

8.3.3 Confounding Factors

As seen with the analysis in Chapters 5-7, this analysis into TWINS-UK data used models of adjustment. The models of adjustment remain the same as with the previous analysis:

- Model 1. No adjustment.
- Model 2. Adjustment for age, BMI, smoking, alcohol consumption, physical activity and HRT.

Gender was not adjusted for in the TWINS-UK analysis, due to it being an all-female subset of data from the cohort.

8.4 TWINS-UK Blood Lipid Results

8.4.1 Analysis investigating Serum Blood Lipids and Radiographic OA

Tables 39-41 show the findings from the analysis of blood lipids and hip, knee and hand OA.

TABLE 39. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND RADIOGRAPHIC HIP OA IN TWINS-UK.

Hip OA	**Model 1** (n=839)		**Model 2** (n=434)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	0.98	(0.34, 2.81)	0.84	(0.21, 3.33)
Apolipoprotein B1	3.15	(1.34, 7.40)**	2.73	0.87, 8.61)
Cholesterol	1.18	(0.89, 1.56)	0.78	(0.53, 1.17)
High Density Lipoprotein	1.04	(0.45, 2.38)	0.51	(0.17, 1.55)
Low Density Lipoprotein	1.07	(0.80, 1.42)	0.73	(0.49, 1.10)
Triglycerides	1.96	(1.28, 3.02)**	2.05	(1.20, 3.51)**

*p<0.05
**p<0.01

TABLE 40. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND RADIOGRAPHIC KNEE OA IN TWINS-UK.

Knee OA	**Model 1** (n=1065)		**Model 2** (n=600)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	0.82	(0.43, 1.57)	0.56	(0.22, 1.40)
Apolipoprotein B1	1.95	(1.11, 3.44)*	0.86	(0.39, 1.88)
Cholesterol	1.25	(1.05, 1.48)*	0.93	(0.73, 1.19)
High Density Lipoprotein	0.63	(0.36, 1.10)	0.71	(0.35, 1.45)
Low Density Lipoprotein	1.26	(1.04, 1.52)*	0.97	(0.75, 1.27)
Triglycerides	1.36	(1.00,1.84)*	0.85	(0.54, 1.32)

*p<0.05
**p<0.01

TABLE 41. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND RADIOGRAPHIC HAND OA IN TWINS-UK.

Hand OA	**Model 1** (n=1218)		**Model 2** (n=690)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	1.19	(0.53, 2.67)	0.78	(0.30, 2.04)
Apolipoprotein B1	4.67	(2.15, 10.16)**	2.67	(1.10, 6.53)*
Cholesterol	1.64	(1.31, 2.05)**	1.31	(0.99, 1.73)
High Density Lipoprotein	0.95	(0.48, 1.89)	0.63	(0.29, 1.38)
Low Density Lipoprotein	1.54	(1.19, 2.00)**	1.15	(0.84, 1.56)
Triglycerides	2.05	(1.37, 3.08)**	1.94	(1.22, 3.08)**

*p<0.05
**p<0.01

Table 39 shows that triglycerides are significantly positively associated with hip OA, across model one (p=0.002) and model two (p=0.009). ApoB1 also shows an initial significant association (p=0.008) with hip OA, although this was lost with adjustment for confounders.

There are no statistically significant associations between blood lipids and knee OA (Table 40) when adjusting for confounding factors, although apoB1 (p=0.020), cholesterol (p=0.012), LDL (p=0.017) and triglycerides (p=0.047) are all positively associated with knee OA initially.

Table 41 shows that triglycerides are consistently significantly positively associated with hand OA, across model one (p=0.001) and model two (p=0.005). It also shows apoB1 to have a statistically significant positive association with hand OA across both model one (p=0.000) and model two (p=0.031). Cholesterol (p<0.001) and LDL (p=0.001) also show an initial statistically significant positive association with hand OA, but these lose statistical significance with adjustment of confounders.

8.4.2 Analysis investigating Serum Blood Lipids and Joint Pain

Tables 42-44 show the findings from the analysis between blood lipids and musculoskeletal pain variables (see Tables 51-54 in Appendix 1 for analysis with additional musculoskeletal pain variables).

TABLE 42. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND KNEE PAIN IN TWINS-UK.

Knee Pain	**Model 1** (n=1791)		**Model 2** (n=593)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	0.99	(0.61, 1.62)	0.99	(0.53, 1.86)
Apolipoprotein B1	1.08	(0.69, 1.70)	0.73	(0.39, 1.36)
Cholesterol	0.99	(0.87, 1.13)	0.85	(0.71, 1.02)
High Density Lipoprotein	0.90	(0.60, 1.35)	1.15	(0.68, 1.94)
Low Density Lipoprotein	0.98	(0.84, 1.13)	0.81	(0.66, 1.00)*
Triglycerides	1.16	(0.91, 1.49)	1.08	0.77, 1.52)

*p<0.05
**p<0.01

TABLE 43. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND HAND PAIN IN TWINS-UK.

Hand Pain	**Model 1** (n=1782)		**Model 2** (n=596)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	1.03	(0.59, 1.78)	0.65	(0.28, 1.49)
Apolipoprotein B1	0.97	(0.60, 1.57)	0.75	(0.35, 1.60)
Cholesterol	1.03	(0.89, 1.19)	0.95	(0.76, 1.20)
High Density Lipoprotein	0.96	(0.60, 1.51)	0.56	(0.28, 1.11)
Low Density Lipoprotein	0.98	(0.83, 1.17)	0.94	(0.73, 1.21)
Triglycerides	1.39	(1.04, 1.86)*	1.51	(0.97, 2.36)

*p<0.05
**p<0.01

TABLE 44. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND CWP IN TWINS-UK.

CWP	**Model 1** (n=1583)		**Model 2** (n=775)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	1.02	(0.45, 2.29)	1.60	(0.68, 3.78)
Apolipoprotein B1	1.61	(0.74, 3.51)	1.13	(0.50, 2.56)
Cholesterol	1.17	(0.93, 1.48)	1.10	(0.86, 1.40)
High Density Lipoprotein	0.54	(0.27, 1.08)	0.81	(0.40, 1.66)
Low Density Lipoprotein	1.16	(0.90, 1.50)	1.06	(0.81, 1.39)
Triglycerides	2.10	(1.34, 3.31)**	1.62	(1.01, 2.60)*

*p<0.05
**p<0.01

Table 42 shows the findings from analysis investigating blood lipids and knee pain, which revealed one statistically significant association between LDL and knee pain (p=0.047). The blood lipid and hand pain analysis (Table 43) did not reveal any consistent statistically significant associations, however, triglycerides shows a positive statistically significant association, in model one (p=0.026), but this is lost with adjustment for confounding factors. Analysis investigating CWP and lipids (Table 44), however, revealed a significant positive association (p=0.001) with triglycerides which remains statistically significant when adjusted for confounding factors (p=0.046).

When investigating additional musculoskeletal pain variables no statistically significant associations were found with back pain. Analysis for neck and shoulder pain revealed one statistically significant (p=0.039) association; apoB1 which remained significant (p=0.033) in model two (Table 52, see Appendix 1). Analysis for elbow and forearm pain (Table 53, see Appendix 1) revealed a statistically significant positive association (p=0.027) with triglycerides when adjusted for confounding factors. Initial significant associations were also found with foot pain. Cholesterol (p=0.025) and triglycerides (p=0.024) were significantly associated with foot pain although this was lost with adjustment for further confounders (Table 54, see Appendix 1).

8.5 Discussion

The investigation into the effects of lipids and the lipid-OA relationship was an approach used in this research, to try and define what is being seen within the dietary data. This analysis found triglycerides to be significantly positively associated with hip OA (p=0.009), hand OA (p=0.005), elbow and forearm (p=0.027) pain (see appendix) and CWP (p=0.046). Associations were also seen with knee OA (p=0.047), hand pain (p=0.026) and foot pain (p=0.024) (see Appendix 1) although these lost statistical significance with adjustment for confounding factors. Cholesterol was also positively associated with hand OA, although did not remain significant in model two (Table 41). HDL was found to be inversely associated with neck and shoulder pain (Table 52, see Appendix 1) and LDL, which was found to be positively associated with knee pain (Table 42).

Triglycerides in particular showed a consistent statistically significant association with hip and hand OA across both models. It is also positively associated with all other pain variables with the exception of knee OA. Despite not replicating the previous findings in EPIC-Norfolk

for knee OA, this analysis has replicated the finding that triglycerides are positively associated with knee pain in females, however this result is not statistically significant. This study replicates findings from a study using the Chingford cohort (that investigated the effects of lipids on hand OA) and found that triglycerides showed an increased risk of hand OA although the findings from this particular study were not significant (Garcia-Gil, Reyes et al. 2017). Triglycerides have been found, through previous Mendelian randomisation studies, to be causal for atherosclerotic cardiovascular disease (Budoff 2016). The Rotterdam study also showed that measures of atherosclerosis are associated with the prevalence of knee and hand osteoarthritis (in women) and the progression of hand OA (Hoeven, Kavousi et al. 2013). Having seen associations between triglycerides and knee OA and knee pain in the EPIC-Norfolk analysis, and with very strong statistically significant positive associations in this chapter also, SNPs that influence triglyceride levels could be a good choice for a Mendelian randomisation approach investigating OA.

With the development of OA, there is greater inflammation and the synovium and cartilage becomes more permeable, allowing lipids in the form of lipoproteins the chance to diffuse through the tissues. Studies have suggested that some lipoproteins have a damaging nature. For example LDL, very low density lipoprotein (VLDL) and its remnants, all of which contain the major protein apoB, which reflects the concentration of lipoproteins likely to contribute towards the formation of fatty deposits within blood vessels (Pischon, Girman et al. 2005).

HDL, which inhibits pro-inflammatory cytokine production (therefore having an anti-inflammatory effect) (Oliviero, Nigro et al. 2012), is of great importance for the transport of lipoproteins and is associated with tissue lipid metabolism and homeostasis. HDL is produced by the combined efforts of apoA1, lipid transporter ABCA1 and plasma enzyme lecithin cholesterol acyltransferase (LCAT) which is produced by the liver and activated, in plasma, by apoA-1. LCAT is necessary for the esterification of free cholesterol, by transferring a fatty-acyl group from lecithin to cholesterol. LCAT acts to convert apoA1 particles into active HDL, after lipidation of apoA-1 by ABCA1. Deficiencies in apoA-1 or LCAT can cause deposits of triglycerides in the liver which can lead to fatty liver disease, suggesting that liver damage could lead to the destruction of cartilage.

Evidence suggests that dysfunctional HDL and LCAT deficiency (resulting in no production of mature HDL) is associated with the development of OA and studies have shown that, in OA patients, serum HDL-C levels are lower than in non-OA cases. Research has also found that *APOA1* negative mice, when fed a western diet, immediately developed OA due to alterations in HDL metabolism (Triantaphyllidou, Kalyvioti et al. 2013). Oxidised LDL has been detected in OA, in human cartilage, and stimulates senescence in chondrocytes by decreasing cell viability. It is known to induce ROS in bovine chondrocytes and reduce proteoglycan synthesis, as well as increasing MMP-3 production in human cartilage. Products from the breakdown of lipids (as a result of ROS), found in the joints of OA patients, have been suggested to contribute towards cartilage degradation and the pathogenesis of OA, by inducing collagen oxidation and cleavage and MMP13 activity.

It is, therefore, not unexpected that HDL shows inverse associations with pain, throughout this analysis, although LDL was found to be inversely associated with knee pain ($p=0.047$) also. ApoB1 is significantly positively associated with OA/pain (with the exception of neck and shoulder pain) especially with hand OA ($p=0.031$) and hip OA ($p=0.008$). The TWINS-UK lipid analysis also shows cholesterol to be positively associated with hand OA ($p=0.000$)

(Table 41) and foot pain (p=0.025) (Table 54, see Appendix 1), although these did not remain statistically significant after adjustment for confounding factors.

Many studies have found that dietary fats influence total cholesterol levels in the blood serum. Saturated fatty acids have been found to increase cholesterol concentration whereas unsaturated fatty acids have been found to decrease cholesterol concentration. That most affected is the level of cholesterol transported by LDL-C and HDL-C for which the levels found in the bloodstream is based on the metabolic events within the liver (Dietschy 1998). Cholesterol fluxes lead to elevated levels of harmful lipids in the circulation, particularly oxidised cholesterol, which leads to ER stress and the resulting response intersects with inflammatory pathways (Chapter 5, Figure 11) (Ertunc and Hotamisligil 2016). Cholesterol is found in this chapter to be positively associated with CWP and (although not significant) is consistent with previous findings. CWP has been found in previous research to be associated with a lack of anti-inflammatory cytokines and these patients within this particular study had high blood cholesterol levels. (Üçeyler, Valenza et al. 2006).

Diets high in ω-3 FAs, such as the Mediterranean style diet, appear to decrease levels of AA and LA and increase levels of EPA in cartilage (Lippiello, Fienhold et al. 1990). While AA has been found to activate mTOR signalling in chondrocytes, DHA appears to be able to reverse this effect (Huang, Wang et al. 2014). Unlike AA, LA, oleic and palmitic acids, ω-3 FAs have an effect on proteinases involved in cartilage matrix degradation. Levels of COX-2, IL-1α, IL-1β and TNFα expression in chondrocytes have been found to decrease (Zainal, Longman et al. 2009).

Changes in the levels of fatty acids in cartilage are also suggested to occur with age in that there are less MUFAs and ω-3 PUFAs and increased saturated fatty acids found in the more mature cartilage (Cleland, James et al. 1995). It has been shown that saturated fatty acids and ω-6 PUFAs independently increased OA severity, scar tissue formation and ossification (Wu et.al., 2014). Figure 14 gives a visual summary of the effects of lipids and fatty acids on the various processes in OA which support the findings presented in this research.

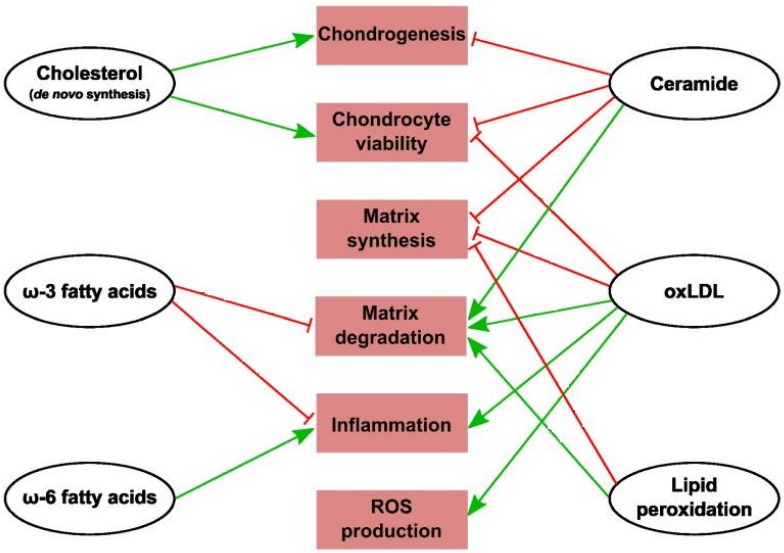

FIGURE 14. SUMMARY OF ACTIONS OF LIPIDS IN CARTILAGE METABOLISM. PROCESSES THAT ARE ACTIVATED ARE REPRESENTED BY GREEN LINES AND PROCESSES THAT ARE INHIBITED ARE REPRESENTED BY RED LINES (VILLALVILLA, GÓMEZ ET AL. 2013).

This cross-sectional analysis of blood lipids in TWINS-UK data, supports previous research and replicates the findings in the initial EPIC-Norfolk cross-sectional analysis. However, more research is still needed to confirm the findings and assess prevalence between populations. The next chapter, therefore, returns to the EPIC-Norfolk cohort to investigate longitudinal pain data, in an attempt to validate the cross-sectional findings throughout this research so far.

9 An Investigation into Dietary and Lifestyle Factors and Change and Incidence of Hip and Knee Pain. A Case-Control Longitudinal Analysis using Data from EPIC-Norfolk.

9.1 Chapter Overview

Having explored the cross-sectional associations between those factors that best predict hip and knee OA and hip and knee pain, in the EPIC-Norfolk cohort in Chapter 3 (and then further exploring the differences in these results based on gender by conducting more specific analyses in Chapter 4), it can be seen that increased age and BMI, having female gender, HRT use and being physically inactive are all positively associated with OA and pain. Dietary components that are particularly high in fats also showed positive associations with hip and knee OA. The TWINS-UK cross-sectional analyses investigated these associations further confirming that a dietary pattern high in fats and lipids, such as cholesterol, LDL and especially triglycerides, are positively associated with OA/pain. Using longitudinal EPIC-Norfolk data from the post-baseline health checks, this chapter will investigate if these factors replicate as being predictive of change and onset of hip pain and knee pain over time. This chapter is a case-control analysis investigating change (both improvement and worsening) of hip and knee pain over time and incidence of hip and knee pain over time. The main findings from this analysis are that BMI is significantly positively associated with incident hip and knee pain, female gender is significantly positively associated with incident hip pain but not knee pain, cholesterol is significantly positively associated with knee pain and increased vegetable consumption is inversely associated with hip pain.

9.2 Introduction

EPIC-Norfolk, as previously discussed, is a large prospective cohort which holds nutritional and lifestyle data alongside clinical OA and pain data of the hip and knee for both males and females, aged 40-79 years of age. Cross-sectional analysis of EPIC-Norfolk data (Chapters 3 and 4) revealed those dietary and lifestyle factors that best predict clinical OA and pain of the hip and knee. The TWINS-UK analysis (Chapters 5-8) investigated the effects of dietary patterns and blood lipids on radiographic OA and musculoskeletal pain, in an all-female cohort. The findings from the cross-sectional analysis in EPIC-Norfolk and TWINS-UK analysis suggest that a diet high in saturated fats, trans-fats and ω-6 PUFAs increases the risk of OA/pain whilst a Mediterranean diet appears protective. Triglycerides are positively associated with knee OA and knee pain in EPIC-Norfolk and this was replicated in TWINS-UK where triglycerides were found to be positively associated with hip, knee and hand OA, joint pain and CWP (although the association with knee OA lost significance after adjusting for confounders). BMI has been found to be consistently associated with OA/pain in the EPIC-Norfolk cross-sectional analysis, however, lifestyle factors such as alcohol consumption and smoking show mixed results. Longitudinal analysis into those lifestyle and dietary factors that best predict OA/pain is needed in an attempt to replicate and validate the findings from the previous chapters. EPIC-Norfolk is a large and sufficiently powered data set and due to the nature of the collection of pain data, pain can be investigated over multiple time points. Previously, the associations between dietary and lifestyle factors and hip and knee pain were investigated at a fixed point in time using baseline nutrition and lifestyle data and pain data from the first health check. However, further analysis is needed to see if these associations remain when the analysis is of a longitudinal design.

This analysis will firstly investigate longitudinal associations between lifestyle and dietary factors and change in hip and knee pain over time. This will, therefore, look specifically at those who experienced pain improvement and pain worsening between the first health check (post-baseline) and second health check and then between the second and third health check. Secondly this chapter will continue to investigate longitudinal associations by looking specifically at those participants that experienced the onset of hip and knee pain between the initial collection of joint pain data at the first health check (post baseline) and the second and third follow-up health checks.

The EPIC-Norfolk cohort, used in such a way as described above, provides the opportunity to investigate which dietary factors alongside lifestyle factors best predict the course of occurrence and recurrence of pain over time. This chapter identifies those explanatory variables that best predict changes in hip and knee pain, however, as this analysis uses baseline dietary data, the assumption when investigating joint pain data from multiple time points is that the diet of the participants in this cohort did not change significantly post-baseline.

9.3 Methods

9.3.1 Statistical Analysis

A logistic regression with a stepwise algorithm to remove non-significant explanatory variables was used to investigate the relationship between independent variables and, firstly, change in hip and knee pain and, secondly, incidence of hip and knee pain.

9.3.2 Independent Variables

This chapter uses EPIC-Norfolk dietary, lipid and lifestyle data outlined previously (please see Chapter 3.3.4, Tables 4-6).Potential explanatory variables included in this analysis are those used previously as in Chapter 3: age, gender, hip circumference, waist circumference, smoking status, teetotal, alcohol intake, ethnic group, physical activity, fruit, leafy vegetables, other vegetables, fatty fish, other fish, chicken, meat, meat products, eggs, cheese, brown bread, milk, snacks, allium vegetables, cruciferous vegetables, green vegetables, yellow vegetables, other vegetables, citrus fruits, non-citrus fruits, chips, energy intake, cholesterol, triglycerides, HDL, LDL and BMI.

9.3.3 Longitudinal Pain Data

The variables relating to pain in the EPIC-Norfolk cohort are knee pain and hip pain and whilst the TWINS-UK subset of data contains much more site specific pain data, it does not include hip pain. This data is therefore very valuable for this research, not only does EPIC-Norfolk include this additional pain variable but it holds this data at multiple time points enabling us to investigate the main predictors and their effects over time.

There is no pain data at baseline in the EPIC- Norfolk cohort. The data used in this analysis was collected at multiple time points: 18 months post baseline (first health check), 3 years post baseline (second health check) and 10 years post baseline (third health check). The

first health check is approximately 18 months after baseline, the second health check is 1.5 years after and the third health check is approximately 3.5 years further after that. The average time elapsed from baseline to the second health check is therefore approximately 3 years and the time from baseline to the third health check is approximately 6.5 years. Table 45 shows the characteristics of EPIC-Norfolk pain data.

TABLE 45. CHARACTERISTICS OF EPIC-NORFOLK PAIN VARIABLES OF INTEREST, AT MULTIPLE TIME POINTS.

	1st Health Check			2nd Health Check			3rd Health Check		
	Pain	**No Pain**	**Missing**	**Pain**	**No Pain**	**Missing**	**Pain**	**No Pain**	**Missing**
Hip Pain	2467	17199	850	1644	13849	5023	1582	12116	6818
Knee Pain	3886	15897	733	2642	12996	4878	2790	11125	6601

Hip and knee pain variables were generated so that they included those who suffered onset of hip and knee pain during the specified time periods (first-second health check and first-third health check). The joint pain variables were coded as binary variables (1= have pain, 2=do not have pain which was recoded 1= have pain, 0=does not have pain for use in statistical software packages). The variable to represent change was also generated as a binary variable (coded: 0='got better having had pain at baseline', 1='got worse (now have pain) compared to baseline').

Those that reported having pain and still had pain at the later time points and those who did not report having pain and responded at the later time points as still not having pain were defined as 'stayed the same'. The analysis selected these randomly by splitting the numbers of those that 'stayed the same' proportionally between these two groups.

9.3.4 Inclusion Criteria

For the analysis investigating the factors that most influence change in pain over time, only those who saw an improvement in joint pain having suffered at baseline and those whose joint pain had worsened since baseline were included. Those who saw no change were excluded from the analysis.

For the analysis investigating the factors that most influence incidence of hip and knee pain over time, only those who experienced the onset of hip and knee pain between time points were included. Those who already had hip and knee pain were excluded.

9.4 EPIC-Norfolk Longitudinal Results

Table 46 shows those explanatory factors that best predict change and onset of hip and knee pain over time.

TABLE 46. SIGNIFICANT PREDICTORS OF CHANGE AND INCIDENCE OF HIP AND KNEE PAIN IN EPIC-NORFOLK OVER TWO TIME PERIODS.

Significant Predictors	**1st-2nd Health Check**	**1st-3rd Health Check**
	OR (95% CI)	**OR (95% CI)**
Change in hip pain	No significant results	
Other vegetables		0.61 (0.46, 0.81)
Change in knee pain		No significant results
Gender	0.76 (0.63, 0.92)	
Cholesterol	1.10 (1.02, 1.20)	
Onset of hip pain		
Gender	1.61 (1.28, 2.03)	1.38 (1.13, 1.69)
BMI	1.07 (1.04, 1.10)	1.06 (1.03, 1.08)
Age		1.02 (1.01, 1.03)
Onset of knee pain		
Age		1.01 (1.00, 1.02)
BMI	1.09 (1.06, 1.12)	1.03 (1.01, 1.05)
Energy Intake	1.00 (1.00, 1.00)	

9.4.1 Change in Hip Pain between Health Checks

Analysis took place to investigate change in hip pain between the health checks, outlined previously. When investigating change in hip pain the number of those who suffered onset of hip pain between the 1st and 2nd health checks was n=670. For those who 'stayed the same' n=13393 and for those who 'got better' n=904, so the total number of observations for absolute change in hip pain was n=1574. A stepwise algorithm was used to remove each variable that was non-significant of which the results were that nothing was significant in the model.

When investigating change in hip pain between the 1st and 3rd health checks, the number of those who suffered onset of hip pain was n=867. For those who 'stayed the same' n=11411 and for those who 'got better' n=958, so the total number of observations for absolute change is n=1825. The results showed that the food group 'other vegetables' is the only significant explanatory factor of hip pain (p=0.0045). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

9.4.2 Change in Knee Pain between Health Checks

Analysis also took place to investigate change in knee pain between the health checks outlined previously. When investigating change in knee pain between the 1st and 2nd health checks, the number of those who suffered onset of knee pain was n=914. For those who 'stayed the same' n=12975 and for those who 'got better' n=1288, so the total number of observations for absolute change is n=2202. The results showed that female gender is significantly inversely associated with knee pain. Being female suggests a decreased risk of knee pain (p=0.0048). Cholesterol is significantly positively associated with change in knee

pain (p=0.016). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

When investigating change in knee pain between the 1st and 3rd health checks, the number of those who suffered the onset of knee pain was n=1379. For those who 'stayed the same' n=10908 and for those who 'got better' n=1229, so the total number of observations is n=2608. A stepwise algorithm was used to remove each variable that was non-significant of which the results were that nothing was significant in the model.

9.4.3 Incidence of Hip Pain between Health Checks

Table 46 shows the results from the investigation of the onset of hip pain, between the health checks (outlined previously). When investigating incidence of hip pain the number for those who suffered onset of hip pain between the 1st and 2nd health checks was n=670. With there being 670 cases of those who suffered onset of hip pain over approximately three years, 670 of the 13393 who 'stayed the same' were randomly selected as controls and those who had previously suffered but no longer had hip pain were excluded. The results showed that strong predictors of onset of hip pain in EPIC-Norfolk are female gender (p<0.0001) which significantly increases the risk of experiencing hip pain compared to being male. BMI (p<0.0001) also increases the risk of experiencing more hip pain. All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

Investigating the onset of hip pain (within the time period of approximately six years), using the same method as above, 867 cases experienced onset of knee pain so 867 non-cases were randomly selected from the 11411 who 'stayed the same'. Those who had previously suffered but no longer had hip pain were excluded. The results showed that strong predictors of hip pain over this time period are female gender (p=0.0016) which significantly increases the risk of hip pain, age (p=0.0028) which significantly increases the risk of hip pain and BMI (p<0.0001), which significantly increases the risk of hip pain. All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

9.4.4 Incidence of Knee Pain between Health Checks

Analysis took place to investigate the onset of knee pain between the health checks outlined previously. When investigating incidence of knee pain those who suffered onset of hip pain between the 1st and 2nd health check was n=914. With there being 914 cases of knee pain, 914 controls were randomly selected from the 12975 who 'stayed the same'. Those who had previously suffered but no longer had knee pain were excluded, creating a total of 1828 participants. The strongest predictors of the onset of knee pain during this period of time are BMI (p<0.001) which significantly increases the risk of onset of knee pain and energy intake (kj) which is significantly associated with the onset of knee pain (p=0.022). All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

Investigating the onset of knee pain within the time period of approximately six years (between 1st and 3rd health check), using the same method, there were 1379 cases so 1379

controls were randomly selected from those who 'stayed the same'. Those who had previously suffered but no longer had knee pain were excluded, creating a total number of 2758. The strongest predictors of the onset of knee pain, during this period of time, were age (p=0.020) which significantly predicts the onset of knee pain and BMI (p=0.002) which also increases the risk of onset of knee pain. All other variables were taken out of the analysis by the stepwise algorithm as they were not significant.

9.5 Discussion

This longitudinal analysis investigating change in pain over time addressed, in part, the previous findings that showed an unexpected positive association between vegetable consumption and risk of OA and pain. This analysis found that increased vegetable consumption is inversely associated with risk of hip pain (over a period of approximately six and a half years) which confirms findings from previous research. A study in TWINS UK investigated the effects of dietary components in OA and identified diallyl disulphide (DADs), found in allium vegetables such as garlic, to be associated with reduced hip OA occurrence. DADs have been found to block expression of key matrix-degrading proteases (Williams, Skinner et al. 2010). This longitudinal analysis, therefore, supports findings from previous research.

The results looking at the longitudinal analysis of onset of hip and knee pain over time confirms that the cases (those that experienced the onset of knee pain) in this study were of older age, higher BMI and female gender suggesting that these all play a key role in the development of pain. These known risk factors for OA confirm the associations seen in the cross-sectional analysis in Chapters 3 and 4, where each of these factors was found to be strongly associated with the risk of both OA and pain.

Female gender was found to be inversely associated with change in knee pain over a three year period however was consistently found to be positively associated with incident hip pain (61% increased risk) over three years and this association remained in the analysis investigating incident hip pain over six and a half years (38% increased risk). The finding that gender plays a significant role in the onset of hip pain supports the previous findings that show that HRT use is positively associated with increased hip/knee pain in middle aged females. Energy intake was found to be associated with incident knee pain, over a three year period. This finding is as expected if it is assumed that the excess calories contribute to weight gain. Therefore, caloric restriction (reducing weight gain) could prevent OA/pain despite caloric restriction in mouse models having not found any protective effects on age related OA (McNeill, Wu et al. 2014).

BMI particularly is consistently positively associated with incident hip and knee pain (in all analysis), however, no association was found between BMI and change in hip and knee pain. There is a lot of research investigating the effects of BMI and weight gain and studies have found body fat distribution to be a strong determinant of insulin resistance and inflammation (Jennings, MacGregor et al. 2017). It is now recognised that adipose tissue plays a role in inflammatory processes and not necessarily just by exerting negative effects by putting additional pressure on the joints, through increased load.

Adipocytes (the main component of adipose tissue) are mainly for storing and releasing lipids but adipose tissue is also now well regarded as an endocrine organ (Scherer 2006). It

is involved in regulating metabolic homeostasis and balancing energy. This leads to the polypeptides secreted by adipocytes, known as adipokines, and fatty acids being regulated.

The signalling effects from adipose tissue also include regulation of satiety and interference of glucose related hormones from the pancreas. There is now also more recognition of communication from other tissues that can change the regulation of adipokines (Stern, Rutkowski et al. 2016). Pro-inflammatory and anti-inflammatory factors are both released from adipose tissue for example leptin, adiponectin, resistin and visfatin. Alongside these, cytokines and chemokines such as TNF-α, IL-6 and monocyte chemoattractant protein 1 are also released (Fantuzzi 2005).

Adipocytokines have been explored specifically in relation to osteoarthritis of the hand which is a more suitable option to investigate how metabolic factors might influence OA due to there being no explanation of mechanical force (Yusuf, Ioan-Facsinay et al. 2011).

The most secreted protein is the adiponectin hormone which is believed to play a role in the pathology of OA and has been heavily investigated in relation to many other diseases and biological pathways. Studies have found plasma adiponectin to be associated with OA severity specifically in knee OA (Cuzdan Coskun, Ay et al. 2015). However, adiponectin has also been found to potentially protect against inflammation, by influencing NF-κB components and downstream transcription factors. Increased serum adiponectin levels have been found to reduce levels of MMP-9 and exogenous adiponectin has been found to increase gene expression of eNOS and IL-10 known to be anti-inflammatory mediators. Exogenous adiponectin has also been found to reduce gene expression of pro-inflammatory factors such as TNF-α and IL-6 (Wang, Chen et al. 2016).

There have been many conflicting studies and the role of adiponectin is still not well understood (Zembala-Szczerba, Jaworowski et al. 2017) but adiponectin's potential uses as a biomarker have been suggested (Cuzdan Coskun, Ay et al. 2015). Adiponectin levels in synovial fluid have been investigated alongside the adipocytokine leptin, also secreted by adipose tissue (Simopoulou, Malizos et al. 2007). It had been suggested that in malnourished individuals reduced leptin levels may increase vulnerability to infection due to the reduced immune response (Fantuzzi 2005).

With regard to OA, it has been shown that a greater adiponectin/leptin ratio is associated with lower levels of pain in patients with severe knee OA (Gandhi, Takahashi et al. 2010). Leptin has also been shown to directly affect chondrocytes (Simopoulou, Malizos et al. 2007) which is unsurprising. Leptin expression is upregulated in tissues surrounding the joint such as cartilage and subchondral bone (which, as already discussed, experience vast structural and biochemical changes as a result of OA) compared with tissues that remain unaffected. Larger amounts of 'free leptin' have been found in the synovial fluid of female OA patients than in males (Pottie, Presle et al. 2006).

Another adipocytokine detected in both the synovial fluid and the plasma within OA patients is resistin (Presle, Pottie et al. 2006). Recent studies have found levels of resistin to be positively associated with damage to the cartilage and with BMLs (Wang, Xu et al. 2015). With adipose tissue being an important regulator of metabolic processes and lipid metabolism it is important to consider the link with and the potential adverse effects of obesity. As a result of over-nutrition there exists stimulation of the release of non-esterified

fatty acids (NEFAs) whereas under-nutrition decreases circulating leptin and increases adiponectin (Stern, Rutkowski et al. 2016).

The other way in which excess adipose tissue may play a key role in the development of OA is through its role in regulating hormones. As discussed in Chapter 1, hand OA may be influenced by hormones and the mechanisms through which adipose tissue may be affecting hormone imbalance.

Hand OA, when experienced post menopause, frequently occurs without other joints being affected (Szoeke, Cicuttini et al. 2008) so hormones could be playing a role in the development of OA. Therefore, another way in which excess adipose tissue could be affecting the progression of OA is through hyperoestrogenism, which relies on the stores of androstenedione in adipose tissue for the formation of oestrogen. Oestrogen is known to play a role in the degradation of the ECM when pharmacological doses of oestrogen are administered. With the evidence throughout the literature it has been suggested that the onset of OA could be related to an incidence of hormone imbalance (Spector and Campion 1989) and from the evidence above it appears, with regard to hand OA specifically, that obesity is a risk factor because of its pro-inflammatory nature rather than the potential mechanical effect. Since many experience menopause with no appearance of joint symptoms it could be that onset of disease is more likely in those who experience fluctuations in hormone levels, alongside other existing risk factors or that these changes could influence the progression and/or symptoms of the disease (Watt 2016).

Reviewing the literature on adipocytokines and discussing how each can affect OA of the hand has shown how metabolic factors produced by the fat tissue are potentially having a direct effect and it is, therefore, suggested that factors associated with obesity are involved in the onset and development of OA (Yusuf, Ioan-Facsinay et al. 2011). Since obesity and female gender are both heavily associated with OA, it is also suggested that females could be predisposed to higher adipokine levels. This could, therefore, explain the increased prevalence of hand OA (Gabay and Gabay 2013) and is consistent with the hand OA and lipid results seen in this thesis within the all-female TWINS-UK cohort.

Cholesterol showed a positive association with change in knee pain ($p=0.016$) over a three year period. Cholesterol and fatty acids are lipids frequently linked to cartilage physiopathology. High cholesterol content is found in chondrocyte cell membranes and is therefore important for structural maintenance. Cholesterol biosynthesis has been found to be essential for chondrogenesis in rats. Liver X Receptor (LXR) is a sensor of oxygenated cholesterol derivatives, which can protect cells from an overload of cholesterol and regulate cholesterol during chondrocyte differentiation.

Another critical regulator of cholesterol metabolism is the mammalian or mechanistic target of rapamycin (mTOR), which regulates the expression of genes such as ABCA1, LXR the LDL scavenger receptor LOX-1. If cholesterol distribution in plasma membranes is altered the mTOR pathway is affected. Inhibition of mTOR by rapamycin has been found to prevent chondrocyte differentiation and has shown decreased severity of OA and less cartilage degradation (Villalvilla, Gómez et al. 2013). Previous studies have shown that high serum cholesterol or hypercholesterolemia is positively associated with knee OA (Stürmer, Sun et al. 1998). Positive associations have also been seen between high serum cholesterol and generalized OA, in knee OA participants. It is believed that serum cholesterol has a role

as a systemic risk factor for OA and that differences seen in OA patterns are likely to be due to adjusting for other risk factors (Stürmer, Sun et al. 1998).

The results from the longitudinal analysis although supporting many of the findings from the cross-sectional analyses do also show some differences from the cross-sectional analysis which calls into question the reliability of cross-sectional observations, discussed further in Chapter 11. Having found consistent associations particularly with BMI the next chapter, therefore, investigates how OA is influenced and addresses the issue of reverse causality.

10 Investigating Cause and Effect. An Analysis of Single Nucleotide Polymorphism Data from the EPIC-Norfolk Cohort.

10.1 Chapter Overview

BMI has been shown throughout cross-sectional and longitudinal analyses to be a strong predictor of OA/pain in the EPIC-Norfolk cohort. Triglycerides were also found to increase the risk of knee pain in EPIC-Norfolk, especially in females, aged 40 to 52 years. Triglycerides then showed consistent associations across models with hip and hand OA a pain variables in the TWINS-UK cross sectional analysis in Chapter 8. Two SNPs that influenced both BMI and triglycerides were therefore requested, from EPIC-Norfolk, to explore reverse causality. A logistic regression analysis was first performed to ensure there was a positive association of each explanatory variable (BMI and triglycerides) with each outcome variable (knee OA and hip OA). A Mendelian Randomisation approach then used SNP rs1421085 from *FTO* and SNP rs662799 from *APOA5* to investigate whether BMI and triglycerides were causal of OA. With the exception of triglycerides and hip OA the explanatory variables showed positive associations with knee and hip OA and analysis between the SNPs and explanatory variables confirmed them as being good instrumental variables. However the analysis with the fitted values from the regression analysis did not show any statistically significant associations. The analysis for this chapter did not, therefore, lead to any 'causal' conclusions for hip or knee OA for either BMI or triglyceride levels.

10.2 Introduction

In epidemiological studies when an association is observed it is then a question of whether that association is one that represents a true cause and effect relationship so if the exposure were to be altered would the frequency of the disease be influenced also? (Willett 2012). Mendelian randomization refers to the event whereby an individual's genotype is randomly assigned from the parental genotypes before conception. The aim of Mendelian Randomisation studies is to build knowledge with regard to gene function, using genotype as a tool for understanding more about the modifiable exposure of interest (Lawlor, Harbord et al. 2008). Single Nucleotide Polymorphisms (SNPs) are the variations in single nucleotides within DNA sequences across the genome resulting in genetic differences between individuals (Klug, Cummings et al. 2009). This variation at a single position in a DNA sequence must occur in at least 1% of individuals to be classed as a SNP. If a SNP occurs within a gene, then the gene is described as having more than one allele which may lead to variations in the amino acid sequence and therefore contribute to a change in the phenotype of individuals. SNPs can therefore be used as Instrumental variables (IVs) in aetiological epidemiology, to investigate associations between genotype and the effects of a modifiable non-genetic exposure on disease outcome. Applying the theory of IV analysis to investigate associations between genotype and the effects of modifiable non genetic exposures on OA deals with endogeneity i.e. confounding, reverse causality and regression dilution bias. In this method the IV variable is associated with the outcome through a strong association with an intermediary variable, a risk factor or exposure, such as BMI. Mendelian Randomization studies therefore use genetic variation as

a proxy for 'environmentally modifiable exposures of interest' or an 'intermediate phenotype' in order to investigate causation (Lawlor, Harbord et al. 2008).

In studies that are seeking to explain the 'causes' of diseases, the method of Mendelian Randomisation uses a SNP or combination of SNPs in a gene (or multiple genes) that are known to be significantly associated with the explanatory variable that appears to be 'causing' the disease of interest. The data used in this thesis are SNPs, identified through GWAS, which act as an IV because they meet the prerequisite for a valid Instrumental variable: the IV induces changes in the explanatory variable (BMI or Triglycerides) and has no significant effect on the dependent variable (OA).

The results, using data from the TWINS-UK cohort, showed associations between a 'Traditional English' diet (a diet high in saturated fats) and OA/pain. In Chapter 8, again using data from the TWINS-UK cohort, results revealed associations between elevated lipid levels (particularly triglycerides) and OA/pain. Both the initial cross-sectional and the longitudinal analyses, in the EPIC-Norfolk cohort, consistently show BMI as a strong predictor of OA and pain. The main finding from the longitudinal analysis found that BMI is consistently associated with incidence of hip and knee pain and is the main predictor of onset of both hip and knee pain over both over the three year time period and the six year time period.

This poses an important research question when considering cause and effect especially when interpreting cross-sectional associations. Is it that people experience weight gain, due to dietary intake, and the onset of OA/pain then occurs or does the onset of OA and subsequent pain cause a shift in dietary eating habits to more palatable foods in order to make themselves feel better? A study that took place in the US, investigated student's eating habits and revealed how eating tasty foods in order to cope is linked to increases in BMI (Boggiano, Wenger et al. 2015). The same question of reverse causality can be applied to increases in circulating lipids. Is it that people eat a particularly fatty diet and experience a rise in lipid levels that causes the onset of OA/pain or is it that people experience pain symptoms and then tend to eat a diet higher in fats as a way of comforting themselves which then results in these negative effects on health? VanDenKerkhof *et al.,* discusses the relationship between pain, diet and lifestyle being confused with regard to those feeling pain having had specific past dietary patterns or whether diet and lifestyle changes were perhaps altered after the onset of pain. Results from this study showed that deterioration of diet was found to be likely in those with CWP, aged 33-42, compared to those of the same age without CWP where diet was found to be stable (VanDenKerkhof, Macdonald et al. 2011).

The main question therefore is could it be that these associations are appearing because people are eating highly palatable foods simply to comfort themselves in order to try and cope with joint pain resulting in increased BMI and elevated lipid levels. Or are these two explanatory variables indeed causing OA? Although OA has been shown to have strong associations with BMI and triglyceride concentrations in the previous chapters, it is as discussed above, still unclear which 'direction' the association is in (i.e. which is 'causal').

It is possible to introduce an Instrumental Variable (IV) in the statistical analysis to assist in defining causal relationships. Therefore, the two SNPs within EPIC-Norfolk chosen to be investigated in this thesis are *FTO* SNP rs1421085 and *APOA5* SNP rs662799. The SNP rs1421085, found within the fat mass and obesity associated gene, is known to be

associated with increased risk of obesity and is just one of the many co-inherited SNPs within the FTO gene, as discussed in Chapter 1. The SNP rs662799, found within *APOA5*, has been found to be associated with triglyceride metabolism (M Forte and O Ryan 2015). These are both the subject of this analysis and by gaining access to additional data through EPIC-Norfolk, there was an opportunity to investigate further what genetic influence exists, how this may contribute towards OA/pain and in which direction associations found throughout this project may occur (Figure 15).

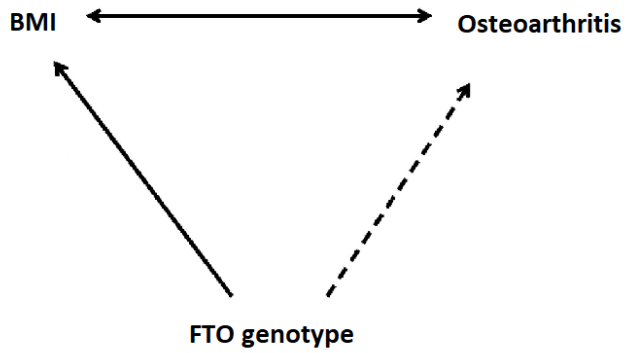

FIGURE 15. THE POTENTIAL DIRECTION OF CAUSALITY BETWEEN BMI AND OA USING *FTO* GENOTYPE AS AN IV.

10.3 Methods

Chapter 3.3.1 and Table 5 (Chapter 3.3.4) describes the HES OA data using for this Mendelian Randomisation analysis.

For an individual in EPIC-Norfolk that has been genotyped for the gene of interest, each SNP will be comprised of two letters (e.g. CC, CT or TT) that are the two possible nucleotides that can occur at that position within the gene. Each of these combination of letters is a genotype and there are therefore three possible genotypes for each SNP. For analysis into the effect of the SNP on the explanatory variable, the three genotypes are labelled either 0, 1 or 2 which reflects their 'effect' on the variable (a 'per allele' effect). As an example, if it is known that the 'C' nucleotide has a greater effect on BMI than the 'T' nucleotide in a particular SNP, the 'CC' genotype would be labelled as '2', the 'CT' as '1' and the 'TT' as '0'. This is done so that the resultant, recoded, ordered SNP data can be used as an explanatory variable in a regression analysis.

The modelling process follows what is known as two-stage least squares regression: stage 1 is fitting the SNP data (as an explanatory variable) to the BMI (the variable that appears to be 'causing' disease) and then using the predicted values from this in stage 2, as the explanatory variable in a logistic regression model with the disease (OA) as the outcome. The significance of the resulting regression coefficient will be taken as evidence for (or against) BMI 'causing' OA.

By using statistical models to help us determine causality, firstly a regression model of the ordered SNP data and BMI was generated. The resulting linear model calculated the values of BMI that minimised the (squared) differences between the actual data and the predicted values. The range (or variance) of these predicted values are the values of BMI that the SNPs can explain. The predicted values were then used as the explanatory variable with OA

as the outcome. If the predicted values are significantly associated with the disease then this provides good evidence that changes in BMI is casual of OA.

10.4 Mendelian Randomisation Results

For the SNP rs1421085 the group with the lowest risk (TT) comprised 5275 participants, 7235 participants are CT and those within the high risk group (CC) comprised 2514. Of the initial 20,517 analysis took place with 15,024 due to 5493 having missing data.

The number of participants with the lowest risk (AA), when investigating SNP rs662799, are much higher and comprised 13,207. 1512 participants had an increased risk (AG) leaving 55 participants in the group with the highest risk (GG). Of the initial 20,267, analysis took place with 14,774 due to 5493 having missing data.

10.4.1 Hip OA

SNPs of interest, were used as IVs, in a Mendelian Randomisation approach to looking at causality of BMI (through the *FTO* gene) and causality of triglycerides (through the *APOA5* gene) with hip OA.

10.4.1.1 *FTO* SNP rs1421085

A logistic regression of hip OA against BMI revealed a very strong positive statistically significant association (OR 1.09, CI (1.08, 1.11), $p<2x10^{-16}$). To determine whether BMI is 'causal' the IV in this analysis is SNP rs1421085 from the FTO gene. In this SNP, the 'C' nucleotide leads to a higher risk of obesity so the SNP was therefore coded TT=0, CT=1, CC=2.

In order to check that the SNP is not a 'weak instrument' a regression analysis for this SNP and BMI took place in which the regression coefficient=0.340 (se=0.044) is highly significant ($p=8.85x10^{-13}$) so the SNP is therefore a good instrument. The fitted values from the regression of BMI against the SNP were then used as the explanatory variable in a logistic regression with hip OA (OR 0.91, CI (0.62, 1.34), p=0.65).

The conclusion is that the evidence (from SNP rs1421085) leads us to believe that BMI is not causing hip OA.

10.4.1.2 *APOA5* SNP rs662799

A logistic regression of hip OA against triglyceride levels revealed an association that was not statistically significant (OR 1.05, CI (0.97, 1.12), p=0.19). Due to there being no significant association triglycerides are therefore not suggested to be 'causal' for hip OA and no further analysis took place.

10.4.2 Knee OA

SNPs of interest, were used as IVs, in a Mendelian Randomisation approach to looking at causality of BMI (through the *FTO* gene) and causality of triglycerides (through the *APOA5* gene) with knee OA.

10.4.2.1 *FTO* SNP rs1421085

A logistic regression of knee OA against BMI revealed a very strong positive statistically significant association (OR 1.14, CI (1.13, 1.16), $p<2x10^{-16}$). To determine whether BMI is 'causal' the IV in this analysis is SNP rs1421085 from the FTO gene. In this SNP, the 'C' nucleotide leads to a higher risk of obesity so the SNP was therefore coded TT=0, CT=1, CC=2.

In order to check that the SNP is not a 'weak instrument' a regression analysis for this SNP and BMI took place in which the regression coefficient=0.340 (se=0.044) is highly significant ($p=8.85x10^{-13}$) so the SNP is therefore a good instrument. The fitted values from the regression of BMI against the SNP were then used as the explanatory variable in a logistic regression with knee OA (OR 1.10, CI (0.77, 1.54), p=0.62).

The conclusion is that the evidence (from SNP rs1421085) leads us to believe that BMI is not causing knee OA.

10.4.2.2 *APOA5* SNP rs662799

A logistic regression of knee OA against triglyceride levels revealed a very strong positive statistically significant association (OR 1.14, CI (1.07, 1.21), $p=6.55x10^{-5}$). To determine whether triglyceride levels are 'causal', the IV in this analysis is SNP rs662799 from the APOA5 gene. In this SNP, the 'G' nucleotide leads to a higher risk of obesity so the SNP was therefore coded AA=0, GA=1, GG=2.

In order to check that the SNP is not a 'weak instrument' a regression analysis for this SNP and triglyceride levels took place in which the regression coefficient=0.265 (se=0.028) is highly significant ($p<2x10^{-16}$) so the SNP is therefore a good instrument. The fitted values from the regression of triglyceride levels against the SNP were then used as the explanatory variable in a logistic regression with knee OA (OR 1.21, CI (0.45, 3.08), p=0.70).

The conclusion is that the evidence (from SNP rs662799) leads us to believe that BMI is not causing knee OA.

10.5 Discussion

Mendelian Randomisation does bring with it statistical challenges and although studies can take place without exclusion criteria or participants consenting to being randomly allocated treatment, studies occur within representative samples within the general population so they are susceptible to the alteration of factors. When investigating the association between genetic variants and disease outcome, alleles are not considered related to confounding factors such as socioeconomic position and other lifestyle factors and so are not included. In addition, genotypes are not affected by the disease process and associations between genotype and disease outcomes are therefore not affected by reverse causality (Lawlor, Harbord et al. 2008).

Within this analysis, the data showed that statistically significant associations existed between BMI and triglycerides and OA proving them to be good instrumental variables to use. Both SNPs satisfied the IV criteria in that the genetic variant is reliably associated with the exposure, in this case overweight status and elevated triglycerides, the exposures are positively associated with disease outcome, there is no direct effect of genotype on disease and that any mediated effect exists through the exposure of interest. Both SNPs, therefore, appeared to be good instrumental variables to use for a mendelian randomisation analysis.

All logistic regression analysese within this data were found to be positive and significant with the exception of SNP rs662799 from the *APOA5* gene which did not show a statistically significant association with hip OA and did not therefore lead to further analysis. The analysis of *APOA5* SNP rs662799 that did take place, however, showed that triglycerides (as explained by this SNP of *APOA5*) are not 'causal' of knee OA. The analysis of the *FTO* SNP rs1421085 in this chapter did not lead to any 'causal' conclusions for BMI (as explained by this SNP of *FTO*) for hip or knee OA.

Published results show that a variant within *FTO* contributes to risk of OA and the association was mediated solely by BMI suggesting that *FTO* plays a role in the casual association found between BMI and OA. The SNP rs8044769 was found in a GWAS to be strongly associated with the risk of developing OA (Consortium 2012). It was recently found to support BMI as having a causal effect on OA using a Mendelian Randomisation approach (Panoutsopoulou, Metrustry et al. 2013), however, a study investigating this same SNP in a Chinese population found that FTO was not associated with susceptibility to OA or BMI of which one of the reasons could be due to differences in heterogeneity between ethnicities (Wang, Chu et al. 2016).

A reason for there not being an association in this study could be due to gender differences in the EPIC-Norfolk cohort.A study published in 2009 concluded that a variant of *FTO* was associated only with males and postmenopausal females (Hubacek, Pitha et al. 2009). With not all females being affected and there being more females than males in the EPIC-Norfolk cohort this could have affected the strength of these findings. As just one of the many co-inherited SNPs within the *FTO* gene (Frayling, Timpson et al. 2007) and perhaps not the SNP that has the strongest association with BMI (as it is suggested that there are other SNPs within *FTO* that show a stronger association with obesity), it is not unexpected that no causal associations have been found. There is therefore a need to investigate the causal relationship that BMI may have with OA further through looking at other SNPs within the *FTO* gene as it contains many polymorphisms that have shown associations in previous studies.

APOA5 specifically has been found to be associated with triglyceride metabolism (M Forte and O Ryan 2015). The SNP rs662799, found within *APOA5*, has been found to be associated with higher levels of total cholesterol, LDL-C and lower levels of HDL-C (Wang, Lu et al. 2016). No causal conclusions were found in this research for the APOA5 variant and OA. Not only could this also be due to selection of this single SNP, associations seen here could be that, due to the low numbers in the EPIC-Norfolk cohort with the high risk genotype, this study is therefore lacking sufficient power. This could therefore be the main reason that in this particular study the selected SNPs from the *FTO* and *APOA5* gene did not lead to any causal conclusions.

11 Conclusions.

11.1 Summary

Obesity/BMI is associated with the development of OA and musculoskeletal pain. Throughout this thesis there is a consistent pattern emerging which shows that a fatty diet, lipids and increasing BMI appear to be strong predictors of OA and musculoskeletal pain. There is also evidence emerging from this project that a Mediterranean dietary pattern is protective of radiographic hand OA and joint pain. To investigate the role of obesity and lipids further in OA, two SNPs from two genes that influence BMI and triglyceride levels were analysed but no causal associations were found. This chapter discusses the main findings, the strengths and the limitations of this research and discusses the future direction of this research.

11.2 Main Findings

The main findings from the cross-sectional analysis using the TWINS-UK cohort were that the dietary pattern that contained the most food items of a fatty nature was positively associated with radiographic hand OA, hand pain, neck and shoulder pain, knee pain and CWP. This finding confirmed what has been reported throughout the literature with regard to health and chronic disease, that a diet higher in fats but lower in omega-3 fats is one of a pro-inflammatory nature.

The most interesting of these results from the TWINS-UK analysis is that with a higher intake the 'Traditional English' dietary pattern there is a 28% increased risk of hand OA ($p=0.025$). This association was seen consistently across both models of adjustment suggesting a dietary pattern consisting largely of fatty foods could be influencing the development of radiographic hand OA and the same positive association was seen with hand pain. One crucial point, throughout the literature, is whether the associations seen between a western diet and OA exists because of the foods consumed resulting in an increase in weight resulting in an increased load on the joint. However there is an argument for foods consumed causing a shift in the inflammatory profile through a biological mechanism of action. With the finding that a 'Traditional English' dietary pattern increases the risk of hand OA, this research helps to confirm the theory that the level of inflammation is affected by a diet high in fats without weight loading on the joint. As discussed the 'Traditional English' dietary pattern is characteristic of the dietary pattern found today in industrialized society. Increased intake of pro-inflammatory saturated fat, ω-6 PUFAs and trans-fatty acids combined with a lack of the anti-inflammatory ω-3 PUFAs are mechanisms through which the associations found may be occurring. In addition elevated levels of these fats in the diet can result in an increase in white adipose tissue and the negative effects due to the release of pro-inflammatory factors are also plausible in explaining this association.

A Mediterranean dietary pattern was created to further investigate the role of dietary patterns on the development of OA/pain. Although this is an area that has been paid attention to within the literature, research to date has focused primarily on knee OA and most of these studies have been of a cross-sectional nature. From observing the studies in

the current literature, this is the first study to investigate the relationship between a Mediterranean dietary pattern and hand OA. The result showed that with a higher intake of the Mediterranean dietary pattern there is a significant decrease in the risk of radiographic hand OA (p=0.027) suggesting a protective effect. This result is in line with the findings from Chapters 5 and 6 and the suggestions of the roles that dietary fatty acids play in the development of hand OA/pain. Protective mechanisms, as discussed, may include the anti-inflammatory effects of ω-3 fatty acids or the contribution of polyphenol compounds which have also been found to have anti-inflammatory effects.

The exploratory analysis in EPIC-Norfolk into the predictors of hip and knee OA and hip and knee pain in, Chapters 3 and 4, found unexpected associations with vegetable food groups and there was also the finding that increased non-citrus fruit consumption is positively associated with knee pain in females when stratified by age. A similar association was then seen in the TWINS-UK dietary analysis, in Chapter 5, with the fruit and vegetable dietary pattern showing a positive association with knee OA (p=0.004).

The significance of increased cruciferous vegetable consumption being associated with increased risk of knee and hip pain contradicts the foundation of healthy eating and the longitudinal data showed that increased 'other' vegetable consumption decreased the risk of hip pain when looking at change in hip pain over time. The reliability of cross-sectional results are therefore called into question and this must be considered when looking at all cross-sectional results.

A possible explanation for these unexpected observations, could be that at the point at which the FFQ was completed, those participants who suffer with severe symptoms were aware of the health message that consumption of cruciferous vegetables could lead to reduced inflammation. Those suffering from OA/pain have most likely tried to find ways of alleviating their symptoms or stop them from getting worse which could have perhaps drawn their attention to fruit and vegetable intake as one of many potential remedies. This could have increased their intake of fruit and vegetable consumption which therefore led to the reporting of higher fruit and vegetable consumption on the FFQs but with OA/pain symptoms also developing over time the results therefore show this as a positive association between fruit and vegetables and OA/pain.

A study in the US investigated the consumption of fruit and vegetable intake and found that a community based fruit and vegetable intervention improved knowledge of intake recommendations and found a decrease in the perception of cost of fruit and vegetables as a barrier (Hendrix, Fischer et al. 2008). There is also therefore the chance that participants in TWINS-UK and EPIC-Norfolk were likely to have agreed to take part in other studies and had therefore gained prior knowledge of the beneficial effects of having a health balanced diet rich in fruit and vegetables. This would also have resulted in an increased intake in fruit and vegetable consumption and could explain the positive associations seen. The differences seen, therefore, between the cross sectional and longitudinal data in this thesis question the reliability of observations seen in cross sectional analysis.

Obesity is an emergent property of a complex system of social psychological physical and biological factors. As a result of lifestyle choices, combined with genetics, obesity is an issue in westernised society. Epidemiological studies consider BMI to be one of the most important risk factors for OA (Simopoulou, Malizos et al. 2007) and the findings from the EPIC-Norfolk analysis in this research support that of previous studies. In the EPIC-Norfolk

analysis BMI appeared consistently throughout all analysese (with the exception of the longitudinal analysis investigating change in pain) as a strong predictor of hip and knee OA and hip and knee pain in both males and females and remained consistent in the longitudinal analysis investigating incident hip and knee pain. It is not unexpected that BMI should be positively associated with hip and knee OA/pain as the joint is under more pressure with increased weight. The pain experience could be as a result of the excess weight or due to the joint undergoing physiological changes as a result of onset of OA which then leads to the pain. As discussed, excess adipose tissue could also be having a negative effect on OA/pain directly through the ability to mediate a number of metabolic processes. It was, therefore, important to investigate further the role of lipids in the development of OA/pain. The initial case-control analysis in EPIC-Norfolk showed a positive association between triglycerides and knee OA and knee pain especially knee pain in females, aged 39.6 to 51.6. Replication of these findings took place in TWINS-UK with the result that Triglycerides were significantly associated with OA/pain particularly hip OA ($p=0.009$), hand OA ($p=0.005$) and CWP ($p=0.046$). Longitudinal analysis in EPIC-Norfolk then showed a positive association between cholesterol and change in knee pain over a three year period ($p=0.016$).

This exploratory analysis in EPIC-Norfolk, in Chapters 3 and 4, also found that being teetotal was positively associated with hip pain which is consistent with the observations in other studies where no alcohol consumption and excessive alcohol consumption tend to be worse than moderate alcohol consumption. However when investigating hip pain in females being teetotal was inversely associated with hip pain so more research is needed in this area to confirm associations. Age, BMI, female gender and energy intake were the biggest predictors of incident knee and hip pain in the longitudinal analysis. When investigating pain data in females based on age BMI and HRT use were found to be associated with increased risk of hip pain whilst increased physical activity was found to be inversely associated with hip pain in females, aged 67.5 to 79.8, which is in line with studies that have suggested there may be benefits of physical activity in later life. The EPIC-Norfolk longitudinal analysese investigating the onset of hip and knee pain over time found that female gender, age and BMI are recurring predictors that significantly increase the risk of knee and hip pain suggesting that these three risk factors have the biggest contribution to the onset and experience of joint pain.

The main message from this research is therefore that a diet high in fats, BMI and lipids are all positively associated with OA/pain. The question as to whether these are causal of OA/pain, however, was also investigated using a Mendelian Randomisation approach. This analysis investigated one SNP from *FTO* and one SNP from *APOA5* two SNPs that have been found to influence BMI and triglyceride metabolism. The SNP from the *FTO* gene, associated with obesity, found that BMI, as explained by this SNP, not to be causal of knee or hip OA. The SNP, associated with triglycerides, from *APOA5* also found triglycerides, as explained by this SNP, not to be causal of knee OA. Further analysis investigating cause and effect is needed to confirm the role of these SNPs in influencing OA through the explanatory factors found in this research.

11.3 Strength, Limitations and Bias

The cross sectional analysis from the TWINS-UK provided many inconsistent results and the cross-sectional analysis from EPIC-Norfolk provided a different message to the TWINS-UK and to the current evidence with regard to lipids for instance HDL is suggested to decrease risk of pain in the TWINS-UK but is suggested to increase risk of hip OA in EPIC-Norfolk. Triglycerides were also found to increase risk of hip and hand OA in the TWINS-UK but the cross sectional analysis in EPIC-Norfolk suggested a decrease in the risk of hip OA, although this did not stay statistically significant across all models. So there remains the question of the reliability of using cross sectional data particularly if there is a low number of observations and therefore a lack of power. This is why discussing dietary results from the cross sectional analysis in this project reviews the overall signals of the data. The main reason for this analysis was to look at the key findings that had strength. Although both the cross-sectional analysis from TWINS-UK and EPIC-Norfolk were inconsistent, looking at both of these analysis combined with the observations seen in the longitudinal study there is an emerging story of a link between obesity/BMI/lipids and OA/pain that suggests there may be many metabolic pathways involved in the onset and development of OA/pain particularly with regard to hand OA. The Mediterranean dietary pattern was also found to be protective of Hand OA and associations between polyphenols and OA/pain were also observed.

Methods for measuring dietary intake have been criticised for being responsible for the correlations and associations seen in cross sectional studies especially when metabolic studies have shown opposing results (Willett 2012). The use of FFQs in both cohorts is a form of information and measurement bias and is a limitation of this research. The use of FFQs as a means of collecting data could also have been improved by the use of food diaries which is more accurate for recording dietary data. Reference measures and biomarkers of dietary intake such as whole body calorimetry, urinary nitrogen excretion and doubly labelled water have confirmed that misreporting not only occurs but is quite commonly seen as under-reporting (Mullaney, O'Higgins et al. 2014).

With the extent of under-reporting increasing with intake it has been suggested that the intakes reported are closer to what are perceived to be the norm than the actual intake (Schoeller 1990). It has also since been suggested, therefore, that every study should include a measure of validity (Black, Prentice et al. 1993). Due to cost and suitability of some of these measures a method based on the ratio of energy intake to basal metabolic rate has been used for some time now to detect mis-reporting of dietary intakes (Goldberg, Black et al. 1991). FFQ data is therefore known to be less accurate than other methods of measuring dietary intake and there appears to be under-reporting of energy intake for those with BMI>30 kg/m² within the EPIC-Norfolk data used in this research. This would result from under-reporting of portion sizes in the FFQ which is a well-known phenomenon affecting the reliability of results. The use of FFQs, therefore, is a limitation due to the mis-reporting typically seen when reporting on energy intake.

A study in the US, published in 2015, found that specific groups were more or less likely to misreport on their diet. Under-reporting for example was associated with female gender, older age, those falling into the overweight or obese categories and those who had been less well-educated. Over-reporting, in complete contrast, was associated with male gender,

younger age, those falling into the underweight category and smokers (compared to those who had never smoked) (Murakami and Livingstone 2015). Another study investigated whether the degree of misreporting of nutrients differed between under-, acceptable and over-reporters and found that in comparison to acceptable reporters absolute intake of protein, potassium and sodium was under-reported in those who under-reported energy intake and over-reported in those who over-report energy intake (Murakami, Sasaki et al. 2012). In the EPIC-Norfolk analysis there is a negative correlation with BMI for energy intake which suggests that as more calories are consumed, there is a decrease in BMI. This could be explained by the under-reporting of food consumption as people put on weight or that they could be dieting. Although using FFQ dietary data in cross sectional analysis may not address the direct relationship between diet, lipids and OA/pain, it does have a substantial contribution.

A major advantage of using the TWINS-UK cohort was the high number of females especially as higher levels of pain have been reported in women (VanDenKerkhof, Macdonald et al. 2011). With regard to selections bias females within the TWINS-UK registry have been proven to be representative of the rest of the UK population with a few differences seen in the weight in monozygotic twins, the prevalence of HRT and current smoking (Andrew, Hart et al. 2001). However, whilst measuring the occurrence of pain in a sample representative of the female population, this analysis gives no information about recurrence or continuing pain in order for a longitudinal approach to be taken.

The EPIC-Norfolk cohort is representative of the rural population in the UK but is not ethnically diverse enough to be representative of the general population, however the fact that it is concentrated within one area means follow up is more easily achieved. Also upon looking closely at the dietary data the majority consume healthy diets which is not truly representative of the general population but those who participate in studies tend to be those naturally take more of an interest and are potentially more knowledgeable about general health. With regard to the EPIC-Norfolk pain data collected over the three time points another factor to consider is the way in which the questions were asked and interpreted e.g. the question 'Have you had hip/knee pain on most days of the last month?'. Ideally this needed to be asked in the same way at each of the health checks so preferably questionnaire based and phrased exactly the same way. This is very important with regard to the reliability of results when comparing across time.

A limitation of this research is the inconsistency of definitions of OA and pain. In the TWINS-UK cohort radiographic OA is recorded by KL score based on x-rays whereas data from HES is clinical OA data diagnosed by GPs and Hospitals based on symptoms. EPIC-Norfolk and most TWINS-UK musculoskeletal pain variables asked about pain within the last month whereas back pain in the TWINS-UK, for example, asked about pain in the last 12 months so the definition of pain differed. Due to the multiple risk factors held responsible for the occurrence and severity of pain, in addition to its highly subjective nature, pain is therefore a very challenging research area. In this research pain is used as an outcome variable because it defines OA but because it is subjective and because of the way in which the pain questions were asked the data available for analysis is limited.

Also worth noting for both cohorts, due to TWINS-UK being all female and EPIC-Norfolk having more females than male participants in this research, is that the age at which OA is most commonly seen is also the age at which menopause occurs. Although pain is being investigated in this project as a proxy for OA it is also very important to keep in mind,

specifically with regard to CWP, the potential confounder that menopause can be associated with fatigue, reduced quality of sleep/sleep deprivation and fluctuations in mood which are all known to enhance feelings of pain (Watt 2016). The relationship between CWP and fatigue, which has also been investigated, has been found to be linked to limited social interactions (Ericsson, Palstam et al. 2016). Studies have taken place based on the theory that those who live in socially isolated areas, as opposed to having busy urban lifestyles with constant distractions, experience the feelings of pain more. This was found to be true of CWP in that those more at risk were those in rural areas with poor health, low mood and who rarely had social interactions (Docking, Beasley et al. 2015). With chronic pain and musculoskeletal pain being influenced by many factors and due to OA pain originating from many of the different changes within the joint, it is very difficult to measure pain assessment and find direct associations with specific mechanisms (Cuzdan Coskun, Ay et al. 2015).

With regard to the analysis it is important that all explanatory variables e.g. all of the dietary data, and the outcome variables e.g. all OA data, was analysed together within the same model to avoid the issues of multiple testing which is where statistical significance is seen due to chance when analysing each variable separately. The stepwise logistic regression, although a model with selected variables input into it and analysed as one test, does involve multiple computer based tests within so it is important to consider that there may be type I error here. In avoiding multiple testing issues there is always a risk of multiple collinearity whereby the effects of one dietary variable mask another (for instance a food group and nutrient within the same analysis) and although this is important to consider when assessing the reliability of results a factor to keep in mind with regard to dietary intake is that a meal contains many different foods and therefore dietary components are not consumed one by one. One way of conducting analysis and correcting for multiple testing could have been to use the Bonferroni correction where the p value is divided by the number of tests in the analysis which adjusts the p value (Armstrong 2014). The issue with this, however, would have been that with such a large number of nutritional variables of interest within the TWINS combined with a very low cut off point for statistical significance there would have been very few if any statistically significant associations to discuss.

A limitation within TWINS-UK is the low number of observations seen with some of the analysis due to missing data. The lower number of participants in the TWINS-UK compared to EPIC-Norfolk and the even lower number of observations for the associations between diet and OA/pain means there is less confidence in the conclusions. For the TWINS-UK analysis (Chapters 5-8) the initial number of observations for the analysis in model one was consistently higher than the number of observations in model two due to participants not having data recorded for confounding factors. All OA and musculoskeletal pain variables suffered a drop in numbers for the dietary pattern analysis in model two. This left a lower number for analysis, however, hand OA (901 observations) CWP (941 observations) and back pain (931 observations) had the highest numbers. The pain variables all experienced a much higher drop in numbers from being over 3000 participants in model one to less than 1000 participants in model two. The same occurred for the numbers of observations for the analysis of a Mediterranean dietary pattern. Hand OA (901 observations) and CWP (941 observations) were left with the highest numbers. The lipid results had the lowest numbers out of all the TWINS cross-sectional analysis (yet all still remained above 450 observations). This drop in numbers seen throughout the TWINS-UK analysis in this thesis means there is a

loss of power for model two however studies have investigated associations with far fewer so there is still strength in these the results.

The benefits of using the EPIC-Norfolk cohort means that there is a much larger sample of approximately 20,000 and despite there being missing data particularly with HRT the numbers remain high and have more statistical power. The incident knee pain analysis between the first and second health check contained 1596, losing only around 200 participants, but this number increased substantially when investigating the time period between the first and third health checks with the total number of participants ending up as 2533, again losing just over 200 participants. There were also a greater number of participants investigating incidence of hip pain between the first and third health checks than there were between the first and second health checks. As this looked at participants over a longer time period (approximately six years as opposed to three years) these are not only better at predicting lifestyle and dietary factors involved in the development of pain (especially when compared to cross-sectional analysese but this study is also sufficiently powered by the increase in numbers which means there is a good chance of avoiding type II error.

11.4 Future Research

Twins are able to provide a natural and unique case control study. In the case of monozygotic twin pairs the corresponding genes, gender, age and upbringing makes using twins advantageous over other epidemiological approaches (Pallister, Spector et al. 2014). Twin registers are valuable as they provide information on the genetics of complex traits and an insight into the genetic epidemiology of disease which allows investigation into the interaction of genotype with gender, age and lifestyle factors and also allows for the study of causes of co-morbidity between traits and disease. Twins are therefore a unique opportunity (Boomsma, Busjahn et al. 2002) especially as there exists problems defining what is normal, due to the high variability between individuals with regard to metabolism, genetic epidemiological studies that are undertaken in the future could benefit greatly from the twin approach (Pallister, Spector et al. 2014). In terms of next steps and future direction it would be good to look at diet and nutrition in the TWINS but instead of using the twins cohort as individuals analysis could be completed using the twins as controls against each other.

A greater focus on symptoms as well as aetiology is needed to reveal clinically relevant therapies for OA and some studies have incorporated a large number of patients who have had a joint replacement in acknowledgement of this. It is clear that genes contribute to the risk of developing OA but studies into the genetics of OA pain are also important. A Genome Wide Association Study that is unbiased would be the best study to investigate causation especially if it takes into account those who have had a joint replacement, like that of the arcOGEN study. Using subjects that have undergone joint replacement, who would have been referred due to the pain and disability OA caused them, acts as a valid measure for joint pain. Joint pain is one of the driving factors behind prescribing joint replacement and there have been few studies examining genomics associated with OA pain, instead much of the focus has been on genomics of OA risk and cartilage loss (Thakur, Dawes et al. 2013) which shows a gap in the research and a need for more research.

With regard to the genetics chapter and use of SNPs, an improvement on the current work would be to include more SNPs from more genes to have 'composite', weighted Instrumental Variables for BMI and triglyceride levels. Due to investigating only two genetic variants the Mendelian Randomisation analysese were not as strong as if there had been multiple variants so future work would therefore be to use multiple genetic variants as IVs to improve strength and statistical power.

Within EPIC-Norfolk, there have been some interesting results with hip and knee pain especially when stratified for age in females. These variables could be investigated further by stratifying for both age and BMI in males and females separately. Further analysis that could take place within EPIC-Norfolk could be to define the different dietary groups (for example healthy vs unhealthy) and then analyse these as potential explanatory variables for hip and knee pain. Future research within EPIC-Norfolk data could also think about how to deal with those participants who had operations for knee/hip pain. They have been included in the EPIC-Norfolk analysis in this project as the majority had operations more than 6.5 years post baseline which is after the third health check. There are also other response variables that would be interesting to look at for knee and hip pain, specifically at the second and third health checks in order to investigate the change in knee and hip pain over time.

Having seen the interesting results emerging between hand OA and BMI/lipids, future work might involve a meta-analysis for hand OA in other data. Due to the protective effect seen between hand OA and a Mediterranean diet and flavonoids it could also be that a randomised controlled trial looking at antioxidants in the role of hand OA would be a meaningful way to investigate these associations further.

With the quantity of TWINS-UK data that has been collected, merged and created e.g. the mediterranean dietary pattern, there is sufficient data for more research to take place. This research provides a starting point for further analysis into OA and pain, particularly of the hand, and contributes to the current knowledge and understanding in the areas of nutrition, OA, pain and causality. Future research would be to gain access to metabolite data from the TWINS Registry which could be investigated to see whether a specific metabolite in a certain pathway may be involved in causing OA/pain. Mendelian Randomisation could then be used to determine the direction of causality between metabolites and OA/pain by selecting SNPs of interest.

With the results from Chapter 7 showing that a Mediterranean diet is protective of hand OA further research is needed in order to replicate and validate this association in other cohorts. The limited dietary data available in EPIC-Norfolk meant that there wasn't enough data to be able to create a Mediterranean dietary pattern and an investigation looking into associations between Mediterranean diet and OA/pain in EPIC-Norfolk could not take place. Dietary data does exist at the time points post baseline but is not available for research purposes. If and when it does become available, it would be useful to request and analyse this data using longitudinal hip and knee pain data over time. In this analysis it is assumed that the diets of participants do not change over time and while it is unlikely that dietary habits would change drastically, this is only an assumption and if the data becomes available in future it would be able to confirm the findings throughout this thesis.

The Norfolk Osteoarthritis Registry (NOAR) contains a sufficient amount of data on the joints of the hand to be able to investigate diet and hand OA more closely. Some of the

participants for NOAR are also within EPIC-Norfolk so where data could be merged together there could then be a new data set available to investigate associations between dietary variables and the joints of the hand. This would be helped if the EPIC-Norfolk nutritional data that was collected at those later time points post-baseline could also be made available as it would become a great source of dietary data for analysis where participants of EPIC-Norfolk overlap with NOAR. Analysis of a Mediterranean dietary pattern could then investigated looking specifically at DIP and PIP joints of the hand which would be a novel and exciting research proposal.

List of Abbreviations

A Disintegrin-like And Metalloprotease with Thrombospondin	ADAMTS
Adenosine Di-phosphate	ADP
American College of Rheumatology	ACR
Apolipoprotein A1	ApoA1
Apolipoprotein B1	ApoB1
Arachidonic Acid	AA
Arthritis Research UK Osteoarthritis Genetics Consortium	arcOGEN
Body Mass Index	BMI
Bone Marrow Lesions	BMLs
Chronic Widespread Pain	CWP
c-Jun N-terminal kinase	JNK
Confidence Interval	CI
C-reactive protein	CRP
Cyclooxygenases	COX
Distal Interphalangeal	DIP
Deoxyribonucleic Acid	DNA
Docosahexaenoic Acid	DHA
Eicosapentaenoic Acid	EPA
Extracellular signal-regulated kinase	ERK
Erucin	ERN
European Prospective Investigation into Cancer	EPIC
Extra Cellular Matrix	ECM
Fatty Acids	FAs
Fat mass and Obesity associated	FTO
Food Frequency Questionnaire	FFQ
Genome-Wide Association Studies	GWAS
Global Burden of Disease	GBD

High Density Lipoprotein	HDL
Hospital Episode Statistics	HES
Hormone Replacement Therapy	HRT
Insulin-like Growth Factor	IGF
Interleukin-1	IL-1
Isothiocyanate	ITC
Janus Kinase Signal Transducers and Activators of Transcription	JAK-STAT
Lecithin Cholesterol Acyltransferase	LCAT
Linolenic Acid	LA
Lipoprotein Lipase	LPL
Low Density Lipoprotein	LDL
Matrix Metalloproteinase	MMP
Manchester definition of CWP	CWP-M
Macrophage-colony stimulating factors	M-CSF
Menopausal Hormonal Therapy	MHT
Messenger Ribonucleic Acid	mRNA
Mitogen-activated protein kinase	MAPK
Monounsaturated Fatty Acids	MUFA
Natural Health Products	NHPs
Nicotinamide Adenine Dinucleotide	NAD
N-methyl-D-aspartate	NMDA
Nuclear Factor-Kappa B	NF-κB
Nuclear factor of activated T cells	NFATc1
Osteoarthritis Research Society International	OARSI
Odds Ratio	OR
Osteoarthritis	OA
Peroxisome Proliferators-Activated Receptor	PPAR
Principle Component Analysis	PCA
Polyunsaturated Fatty Acids	PUFA

Psoriatic Arthritis	PsA
Randomised Controlled Trial	RCT
Receptor Activated NF-κB Ligand	RANKL
Recombinant DNA	rDNA
Research on Osteoarthritis against Disability	ROAD
Reactive Oxygen Species	ROS
Short Chain Fatty Acids	SCFAs
Single Nucleotide Polymorphism	SNP
Silent Information Regulator	SIR
Sirtuin	SIRT
Sulforaphane	SFN
Transforming Growth Factor	TGF
Transient Receptor Potential Vanilloid	TRPV
Tumor Necrosis Factor	TNF
Vitamin D Receptor	VDR
World Health Organisation	WHO
Years Lived with Disability	YLD

Glossary

Case–control Studies

A case-control study is designed to help determine if an exposure is associated with an outcome firstly by identifying the cases (a group known to have the outcome) and the controls (a group known to be free of the outcome). This type of study traces back to investigate exposures to learn which subjects in each group had the exposure(s) and compares the frequency of the exposure in the case group to the control group.

Confidence Interval

A confidence interval refers to the amount of uncertainty associated with a sample population estimate of a true population. A 95% confidence interval is a range of values that you can be 95% certain contains the true population value.

Confounding

In epidemiological analysis, confounding is a form of bias. It is a variable that is associated with the exposure of interest and the outcome. The effect should be taken into account to avoid a false relationship being thought to exist between exposure and outcome.

Cross-sectional studies

Cross-sectional studies sample distributions of healthy and diseased subjects in the population at one point in time.

Incidence and Incidence Rate

Incidence is the number of new events (for example, number of new cases of a disease) that occur during a specified period in a population at risk. Incidence rate is incidence divided by the sum of the length of time each individual was exposed to the risk.

Logistic Regression

Logistic regression is the appropriate regression analysis to conduct when the dependent variable is dichotomous (binary). Like all regression analyses, the logistic regression is a predictive analysis. Logistic regression is used to describe data and to explain the relationship between one dependent binary variable and one or more nominal, ordinal, interval or ratio-level independent variables.

Mendelian Randomisation

Mendelian randomisation is a study design that uses genetic variants as instrumental variables (a variable that is associated with the explanatory variable but not with the outcome variable and is independent of the confounding factors of the association between explanatory and outcome variable) to test the causal effect of a risk factor on a disease or health related outcome.

Odds Ratio

The odds ratio is a ratio of two odds where the individual odds that appear in the ratio are usually for an experimental group and a control group, or two different demographic groups.

Prevalence

Prevalence is the fraction of a population that has the disease and is the total number suffering from a disease at a given time.

Principle Component Analysis

A dimension-reduction tool that can be used to reduce a large set of variables to a small set that still contains most of the information in the large set. It involves a mathematical procedure that transforms a number of (potentially) correlated variables into a smaller number of uncorrelated variables called principal components.

Prospective longitudinal study

A study based on observations over the same subjects for a given period. Incidence is investigated via prospective studies/longitudinal studies/follow-up studies. Whichever word is used, these studies monitor a population for a time to track the transition of non-cases into cases.

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13 Appendix 1. Dietary Exposures and Musculoskeletal Pain.

Below are the results from the analysis for other musculoskeletal sites that although relevant when looking at OA are less commonly diagnosed as a site at which OA occurs. Below are the tables from the analysis within TWINS-UK looking at associations between dietary exposures and musculoskeletal pain.

13.1 TWINS-UK Dietary Patterns and Other Musculoskeletal Pain

Below are the results from the analysis between the dietary patterns and back pain, neck and shoulder pain, elbow and forearm pain and foot pain.

TABLE 47. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND BACK PAIN IN TWINS-UK.

Back Pain	**Model 1 (n=1943)**		**Model 2 (n=931)**	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	0.88	(0.75, 1.03)	0.83	(0.67, 1.02)
High Alcohol	0.98	(0.79, 1.21)	1.00	(0.72, 1.38)
Traditional English	0.97	(0.77, 1.21)	1.01	(0.76, 1.34)
Dieting	1.09	(0.86, 1.38)	1.14	(0.84, 1.54)
Low Meat	1.28	(0.97, 1.69)	1.31	(0.90, 1.90)

*p<0.05
**p<0.01

TABLE 48. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND NECK AND SHOULDER PAIN IN TWINS-UK.

Neck and Shoulder Pain	**Model 1 (n=3469)**		**Model 2 (801)**	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	0.98	(0.91, 1.06)	1.05	(0.95, 1.16)
High Alcohol	0.94	(0.84, 1.05)	1.05	(0.89, 1.25)
Traditional English	1.16	(1.04, 1.30)*	1.15	(1.00, 1.31)
Dieting	0.97	(0.85, 1.10)	1.03	(0.87, 1.22)
Low Meat	0.99	(0.86, 1.15)	1.04	(0.85, 1.27)

*p<0.05
**p<0.01

TABLE 49. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND ELBOW AND FOREARM PAIN IN TWINS-UK.

Elbow and Forearm Pain	Model 1 (n=3380)		Model 2 (n=765)	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	0.98	(0.89, 1.08)	0.94	(0.82, 1.07)
High Alcohol	0.96	(0.84, 1.10)	1.01	(0.82, 1.25)
Traditional English	1.06	(0.93, 1.20)	1.11	(0.93, 1.33)
Dieting	1.06	(0.91, 1.23)	1.17	(0.95, 1.44)
Low Meat	1.17	(0.99, 1.39)	1.12	(0.87, 1.43)

*p<0.05
**p<0.01

TABLE 50. OR AND 95% CI FOR ASSOCIATIONS BETWEEN DIETARY PATTERNS AND FOOT PAIN IN TWINS-UK.

Foot Pain	Model 1 (n=3302)		Model 2 (n=741)	
Dietary Pattern	**OR**	**95% CI**	**OR**	**95% CI**
Fruit and Vegetable	1.01	(0.93, 1.10)	1.02	(0.91, 1.16)
High Alcohol	0.97	(0.86, 1.09)	0.93	(0.76, 1.15)
Traditional English	1.05	(0.93, 1.17)	0.98	(0.83, 1.16)
Dieting	0.95	(0.83, 1.09)	1.06	(0.87, 1.30)
Low Meat	1.05	(0.90, 1.22)	1.02	(0.80, 1.30)

*p<0.05
**p<0.01

13.2 TWINS-UK Lipids and Other Musculoskeletal Pain

Below are the results from the analysis between serum blood lipids and back pain, neck and shoulder pain, elbow and forearm pain and foot pain.

TABLE 51. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND BACK PAIN IN TWINS-UK.

Back Pain	Model 1 (n=800)		Model 2 (n=450)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	2.03	(0.74, 5.58)	2.65	(0.79, 8.88)
Apolipoprotein B1	0.66	(0.26, 1.66)	0.38	(0.12, 1.22)
Cholesterol	0.91	(0.70, 1.19)	0.84	(0.61, 1.14)
High Density Lipoprotein	0.64	(0.29, 1.40)	0.79	(0.33, 1.88)
Low Density Lipoprotein	0.90	(0.67, 1.20)	0.83	(0.58, 1.17)
Triglycerides	1.03	(0.63, 1.67)	0.87	(0.47, 1.61)

*p<0.05
**p<0.01

TABLE 52. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND NECK AND SHOULDER PAIN IN TWINS-UK.

Neck and Shoulder Pain	Model 1 (n=900)		Model 2 (n=600)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	0.68	(0.41, 1.14)	0.76	(0.39, 1.51)
Apolipoprotein B1	0.61	(0.38, 0.97)*	0.47	(0.23, 0.94)*
Cholesterol	0.90	(0.78, 1.05)	0.88	(0.72, 1.08)
High Density Lipoprotein	0.78	(0.51, 1.20)	0.78	(0.45, 1.38)
Low Density Lipoprotein	0.89	(0.76, 1.05)	0.84	(0.67, 1.06)
Triglycerides	1.00	(0.77, 1.30)	1.14	(0.80, 1.62)

*p<0.05
**p<0.01

TABLE 53. OR AND 95% CI FOR ASSOCIATIONS BETWEEN BLOOD LIPIDS AND ELBOW AND FOREARM PAIN IN TWINS-UK.

Elbow and Forearm Pain	Model 1 (n=800)		Model 2 (n=550)	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	0.76	(0.41, 1.40)	0.67	(0.30, 1.50)
Apolipoprotein B1	0.73	(0.41, 1.31)	0.83	(0.37, 1.86)
Cholesterol	0.90	(0.76, 1.08)	0.93	(0.74, 1.18)
High Density Lipoprotein	0.64	(0.38, 1.08)	0.55	(0.27, 1.11)
Low Density Lipoprotein	0.91	(0.75, 1.10)	0.91	(0.70, 1.17)
Triglycerides	1.29	(0.95, 1.74)	1.58	(1.05, 2.37)*

*p<0.05
**p<0.01

Table 54. OR and 95% CI for associations between blood lipids and foot pain in TWINS-UK.

Foot Pain	**Model 1 (n=800)**		**Model 2 (n=500)**	
Lipid	**OR**	**95% CI**	**OR**	**95% CI**
Apolipoprotein A1	0.88	(0.50, 1.56)	1.51	(0.61, 3.74)
Apolipoprotein B1	1.70	(1.02, 2.83)	1.39	(0.61, 3.17)
Cholesterol	1.19	(1.02, 1.38)*	1.12	(0.88, 1.42)
High Density Lipoprotein	0.70	(0.43, 1.11)	0.86	(0.42, 1.76)
Low Density Lipoprotein	1.17	(0.99, 1.39)	1.08	(0.82, 1.43)
Triglycerides	1.39	(1.04, 1.85)*	1.39	(0.83, 2.32)

*p<0.05

**p<0.01

14 Appendix 2. Tables of TWINS-UK FFQ Food Groups and Items for PCA Derived Dietary Score Patterns.

Food Items (n = 3262): Distribution of Consumption and Factor Loadings for the First 5 Dietary Patterns With Bootstrapped 95% Confidence Intervals*

Food item	% consuming any	Servings/wk mean (*SD*)	Factor loading with bootstrapped 95% CI for the first 5 dietary patterns (% variance explained by each pattern shown next to pattern name)									
			Fruit and vegetable (8.2%)		High alcohol (3.9%)		Traditional English (3.6%)		Dieting (3.3%)		Low meat (3.2%)	
Baked beans	75.5	1.1 (1.3)	−.01	(−.04, .01)	−.07	(−.18, .10)	.17	(−.01, .25)	.10	(−.19, .36)	**.31**	(−.16, .37)
Beefburgers	16.4	0.1 (0.3)	−.14	(−.16,−.13)	.13	(−.11, .23)	.18	(−.02, .25)	.15	(−.02, .23)	.08	(−.18, .19)
Beer	35.7	0.9 (2.7)	−.03	(−.05,−.01)	**.22**	(.07, .26)	.00	(−.18, .17)	.09	(−.03, .16)	.04	(−.12, .14)
Berries	79.4	0.7 (1.0)	.16	(.14, .19)	−.05	(−.11, .04)	−.05	(−.11, .04)	.00	(−.10, .09)	−.05	(−.13, .06)
Butter	47.1	3.1 (6.3)	−.07	(−.09,−.05)	.11	(.00, .19)	−.03	(−.16, .13)	**−.35**	(−.40,−.03)	**−.20**	(−.40, .37)
Citrus fruit	83.5	3.3 (4.3)	.19	(.16, .21)	−.11	(−.16,−.01)	−.01	(−.12, .09)	.08	(−.11, .16)	−.07	(−.15, .06)
Coffee	75.2	11.3 (12.3)	−.01	(−.04, .01)	.17	(.04, .21)	−.01	(−.17, .14)	.13	(−.20, .23)	−.16	(−.25, .06)
Cooked potatoes	96.9	3.6 (2.6)	.03	(.00, .05)	−.19	(−.27, .00)	.19	(−.02, .28)	−.08	(−.17, .08)	−.02	(−.15, .15)
Crisp bread	38.7	1.2 (3.7)	.11	(.09, .14)	−.06	(−.11, .02)	−.03	(−.10, .05)	.03	(−.07, .10)	.02	(−.08, .11)
Dairy products; high fat	97.6	5.4 (5.4)	−.07	(−.09,−.06)	.14	(.00, .20)	−.13	(−.21, .02)	−.12	(−.20, .02)	−.07	(−.18, .16)
Dairy products; low fat	93.6	6.6 (4.6)	.15	(.13, .17)	**−.29**	(−.33,−.06)	−.05	(−.24, .21)	.25	(−.09, .29)	−.02	(−.27, .22)
Drinks; other	73.7	6.1 (9.5)	.04	(.02, .06)	−.07	(−.14, .07)	−.10	(−.17, .02)	.17	(−.01, .23)	.11	(−.19, .23)
Eggs	86.3	1.4 (1.4)	.00	(−.02, .02)	.04	(−.08, .12)	.11	(.01, .17)	−.05	(−.14, .07)	−.06	(−.16, .11)
Fried fish	52.4	0.4 (0.6)	−.11	(−.13,−.09)	−.02	(−.18, .13)	**.21**	(.08, .24)	−.01	(−.11, .10)	.03	(−.07, .13)
Fried potatoes	88.8	1.7 (1.4)	**−.21**	(−.22,−.19)	.09	(−.17, .23)	**.22**	(.04, .27)	.01	(−.12, .17)	.12	(−.05, .19)
Fruit juice	80.0	3.2 (4.1)	.09	(.07, .12)	.06	(−.03, .12)	−.07	(−.14, .03)	.08	(−.08, .13)	−.04	(−.13, .07)
High fat breakfast cereals	70.0	2.8 (3.2)	.06	(.04, .09)	**−.23**	(−.29, .04)	−.14	(−.26, .12)	.13	(−.17, .23)	−.13	(−.27, .08)
Lasagne	43.2	0.3 (0.4)	.01	(−.01, .03)	.13	(.02, .18)	.00	(−.14, .13)	**.25**	(−.00, .31)	.13	(−.27, .29)
Legumes	98.1	3.7 (2.8)	.19	(.16, .21)	.03	(−.24, .23)	**.29**	(.10, .33)	−.16	(−.27, .18)	.13	(−.09, .28)
Low fat breakfast cereals	32.7	1.2 (2.4)	−.07	(−.09,−.05)	−.06	(−.11, .01)	.05	(−.04, .11)	−.05	(−.12, .10)	.07	(−.03, .14)
Low fat spread	27.9	2.3 (6.1)	.02	(−.01, .04)	−.10	(−.14,−.01)	.02	(−.09, .12)	.15	(−.01, .22)	.11	(−.18, .22)
Margarine	26.6	1.5 (4.5)	−.09	(−.11,−.07)	−.01	(−.08, .07)	.07	(−.01, .12)	.00	(−.12, .16)	.13	(−.04, .20)
Meat	87.4	2.2 (1.8)	−.10	(−.12,−.08)	−.02	(−.27, .21)	**.34**	(.11, .41)	.05	(−.35, .31)	**−.32**	(−.39, .02)
Nuts	51.0	0.8 (1.8)	.06	(.02, .09)	.14	(−.02, .24)	−.15	(−.25, .01)	−.12	(−.20, .11)	.07	(−.09, .18)
Oily fish	76.1	0.9 (1.2)	.15	(.12, .19)	.01	(−.04, .07)	−.01	(−.09, .07)	.02	(−.16, .13)	−.12	(−.17, .02)
Other fish and seafood	79.2	1.0 (1.1)	.18	(.16, .21)	.07	(−.03, .13)	.07	(−.06, .18)	−.04	(−.25, .19)	**−.20**	(−.26, .07)
Other fruit	98.3	12.5 (9.9)	**.27**	(.25, .29)	−.21	(−.26, .00)	−.07	(−.21, .13)	.09	(−.06, .14)	−.01	(−.13, .10)
Pizza	63.5	0.6 (0.7)	−.06	(−.08,−.04)	.15	(.02, .22)	−.10	(−.21, .07)	.18	(−.09, .31)	**.22**	(−.20, .32)
Polyunsaturated margarine	57.3	4.7 (7.4)	−.02	(−.04, .00)	−.08	(−.14, .03)	−.06	(−.15, .06)	.07	(−.10, .21)	.18	(−.10, .25)

Food Items (n = 3262): Distribution of Consumption and Factor Loadings For the First 5 Dietary Patterns With Bootstrapped 95% Confidence Intervals*

Food item	% consuming any	Servings/wk mean (*SD*)	Factor loading with bootstrapped 95% CI for the first 5 dietary patterns (% variance explained by each pattern shown next to pattern name)									
			Fruit and vegetable (8.2%)		High alcohol (3.9%)		Traditional English (3.6%)		Dieting (3.3%)		Low meat (3.2%)	
Porridge	32.4	0.8 (1.8)	.08	(.06, .10)	−.13	(−.17, −.03)	.05	(−.08, .15)	−.14	(−.20, .05)	.00	(−.15, .17)
Poultry	93.3	1.9 (1.3)	.05	(.03, .07)	−.02	(−.13, .09)	.14	(.01, .25)	.17	(−.30, .32)	**−.25**	(−.33, .04)
Processed meats	89.6	2.6 (2.3)	−.10	(−.12,−.08)	.02	(−.25, .24)	**.32**	(.10, .38)	.13	(−.23, .28)	−.16	(−.27, .06)
Salad dressing high fat	68.8	1.6 (2.2)	.12	(.09, .14)	**.30**	(.08, .34)	−.11	(−.31, .13)	−.02	(−.10, .08)	−.03	(−.10, .07)
Salad dressing low fat	39.4	0.7 (1.5)	.14	(.10, .17)	−.02	(−.13, .11)	.10	(−.03, .19)	.18	(−.03, .27)	.13	(−.20, .25)
Savoury pies	38.6	0.3 (0.5)	−.19	(−.21,−.17)	.03	(−.20, .19)	.24	(.09, .27)	.00	(−.10, .09)	−.01	(−.09, .09)
Savoury snacks	83.0	2.5 (3.4)	−.10	(−.12,−.08)	.12	(.03, .17)	−.03	(−.12, .08)	.04	(−.07, .13)	.09	(−.07, .15)
Seasonings	94.9	3.9 (4.0)	.02	(.00, .05)	.05	(−.05, .11)	.07	(−.04, .14)	.02	(−.15, .23)	.18	(−.06, .25)
Soda; high sugar	38.1	1.0 (3.0)	−.08	(−.10,−.06)	.09	(−.01, .15)	.04	(−.07, .12)	.08	(−.05, .19)	.11	(−.12, .20)
Soda; low sugar	46.6	2.3 (5.3)	.02	(−.01, .04)	.06	(−.05, .14)	.04	(−.10, .18)	**.33**	(−.05, .36)	.08	(−.34, .33)
Soup	68.0	1.0 (1.6)	.12	(.10, .14)	−.03	(−.10, .03)	.03	(−.05, .11)	.05	(−.17, .18)	−.13	(−.20, .02)
Soy and other milk	1.3	0.0 (0.5)	.05	(.03, .08)	.00	(−.06, .08)	−.03	(−.11, .05)	−.15	(−.22, .12)	.08	(−.10, .21)
Soy foods	11.2	0.2 (0.9)	.11	(.07, .14)	.06	(−.06, .17)	−.09	(−.24, .05)	−.15	(−.37, .40)	.38	(.03, .43)
Spirits and liquor	52.0	1.4 (3.2)	.00	(−.02, .02)	**.20**	(.06, .23)	.01	(−.17, .16)	.10	(−.18, .20)	−.13	(−.21, .04)
Sweet baked	95.2	8.9 (9.8)	−.16	(−.17,−.14)	**−.22**	(−.26, .00)	−.10	(−.25, .14)	**−.22**	(−.27, .10)	.00	(−.20, .24)
Sweets and sweet condiments	97.3	12.8 (14.9)	−.17	(−.19,−.15)	−.02	(−.08, .05)	−.01	(−.13, .09)	**−.28**	(−.29, .17)	.09	(−.19, .28)
Tea	86.9	20.4 (14.2)	−.03	(−.05,−.01)	**−.23**	(−.28,−.05)	.08	(−.13, .26)	**−.24**	(−.29, .12)	.02	(−.22, .27)
Vegetables; allium	93.2	4.4 (4.0)	**.23**	(.21, .25)	**.27**	(.04, .30)	.06	(−.18, .23)	−.13	(−.18, .07)	.02	(−.13, .18)
Vegetables; cruciferous	98.1	5.9 (4.7)	**.21**	(.17, .24)	−.03	(−.32, .26)	**.36**	(.14, .39)	−.08	(−.20, .15)	.07	(−.08, .19)
Vegetables; green leafy	95.3	3.3 (2.9)	**.30**	(.28, .32)	.12	(−.04, .17)	.07	(−.06, .15)	−.06	(−.12, .10)	.07	(−.04, .13)
Vegetables; other	98.4	6.6 (5.7)	**.32**	(.30, .33)	.18	(−.09, .26)	.15	(−.05, .25)	−.13	(−.20, .15)	.12	(−.07, .19)
Vegetables; yellow	99.5	6.1 (4.2)	**.30**	(.28, .32)	−.03	(−.17, .13)	.18	(.06, .23)	−.09	(−.15, .11)	.06	(−.07, .15)
White and brown bread, refined grains	98.6	11.3 (9.7)	−.11	(−.13,−.09)	.04	(−.02, .09)	−.03	(−.10, .05)	−.02	(−.14, .17)	.15	(−.04, .22)
Wholemeal bread and grains	79.7	5.3 (7.4)	.15	(.12, .17)	−.11	(−.22, .12)	−.18	(−.23, .00)	−.01	(−.10, .08)	−.01	(−.10, .08)
Wine	74.5	3.0 (5.0)	.08	(.06, .10)	**.33**	(.07, .37)	−.14	(−.35, .14)	.03	(−.25, .21)	**−.22**	(−.27, .02)

Note: Figures in bold are loadings ≥ .2 or ≤ −.2

*Bootstrapped confidence interval derived from 10,000 replications using a single, randomly selected member of each twin pair.

FIGURE 16. FFQ DATA TABLES FOR PCA DERIVED DIETARY PATTERNS INSERTED FROM TEUCHER, B., ET AL. (2007). "DIETARY PATTERNS AND HERITABILITY OF FOOD CHOICE IN A UK FEMALE TWIN COHORT." TWIN RESEARCH AND HUMAN GENETICS 10(05): 734-748.