Vitamin D, light exposure, sleep and musculoskeletal health in South Asian and Caucasian women: biological and social influences

Andrea Lisa Darling

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Department of Nutritional Sciences
School of Biosciences and Medicine
Faculty of Health and Medical Sciences
University of Surrey

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Abstract

There is an urgent need to better understand the problem of vitamin D deficiency, and its health effects, in population groups of different ethnicity. The principal aim of this project was to examine vitamin D status, sunlight exposure, and health outcomes in UK dwelling South Asian and Caucasian women. A cohort of 80 postmenopausal and 32 premenopausal South Asian and Caucasian women were assessed for vitamin D status (serum 25-hydroxyvitamin D; 25(OH)D), musculoskeletal health, light exposure and sleep-wake cycles.

In postmenopausal women, South Asians had a significantly lower vitamin D concentration than Caucasians (p=0.002), with 83% of Asians vs. 24% of Caucasians below 50nmol/l for 25(OH)D. Despite adaptations in tibial bone structure of the South Asians to improve bone strength, their bones were weaker by 38% compared with Caucasians (p<0.001). Stand-to-walk time (Asian mean (±SD) time 8.1 s ± 1.8 vs. Caucasian mean (±SD) time 6.9 s ± 1.4); p=0.002) and grip strength (Asian strength=70% of Caucasian strength, p<0.001) were worse in the South Asians.

For both premenopausal and postmenopausal women, Caucasians showed a significantly higher actigraphic sleep efficiency (p<0.001) and lower sleep fragmentation (p=0.002) than Asians. There was a higher outdoor light exposure (over 1000 lux) in premenopausal and postmenopausal Caucasians than in same-age Asians (p=0.052). Qualitative analysis of interview data suggested that religious and cultural influences on family, work and community life may partly explain the reduced sunlight exposure in South Asian women, which contributes to vitamin D deficiency.

The implications of this work are that older South Asian women are in need of intervention to improve vitamin D status. There is also some evidence for poorer musculoskeletal health, lower light exposure and poorer sleep in this group. The qualitative research included in the current study offers future intervention options to improve the health of UK dwelling South Asian women.
Declaration of originality

“This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service TurnitinUK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.”

A.L.Darling
Statement of contribution

General contributions:

General PhD supervision and proofreading of Thesis manuscript: Professor Susan Lanham-New, Professor Sara Arber, Professor Debra Skene and Dr. Kathryn Hart.

The PTH and 25(OH)D analyses were conducted by Dr. Jacqueline Berry from the Specialist Assay Laboratory (Vitamin D) and Manchester Academic Health Sciences Centre, Manchester Royal Infirmary.

Collection of blood from participants, and processing of blood samples were undertaken by Mrs Sue Starkey.

Specific chapter contributions:

Chapter 3

The serum CTX bone markers were measured by Professor Richard Eastell and Ms Fatma Gossiel, Bone Biochemistry Laboratory, University of Sheffield

Dr. Sig. Johnsen conducted the non-linear mixed modelling procedures in sections (3.2.2-3.3.5) and the power analysis in section (3.4.3).

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Chapter 4

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Chapter 5

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1 all University of Surrey staff or students unless otherwise stated
Dr. Peter Lloyd Morgan assisted with the production and editing of the light threshold data from the Actiwatch readings. However, all subsequent statistical analysis and production of figures for the light threshold data, as well as the daily light time profiles, were conducted by myself.

Dr. Thomas Kantermann assisted with the use of the correct usage of the Munich Chronotype Questionnaire.

Dr. Benita Middleton conducted the lux calibration tests for the Actiwatches, as well as assistance with training in use of Actiwatches and proofreading of chapter 5.

Mr. Peter Williams designed the SAS macro for the hourly lux values.

Chapter 6

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Abbreviations

1,25(OH)₂D 1,25-dihydroxyvitamin D
1,24,25(OH)₃D 1,24,25-trihydroxyvitamin D
24,25(OH)₂D 24,25-dihydroxyvitamin D
25(OH)D 25-hydroxyvitamin D
aBMD areal Bone Mineral Density
ALTM All laboratory trimmed mean
ANCOVA Analysis of Covariance
ANOVA Analysis of Variance
AWL Actiwatch
AWL-L Actiwatch with integrated lux metre
BMC Bone Mineral Content
BMI Body Mass Index
BMU Bone Remodelling Unit
BST British summer time
BUA Broadband Ultrasound Attenuation
CAQDAS Computer Aided Qualitative Data Analysis
CLOCK Circadian Locomotor Output Cycles Kaput gene
CoA Cortical Area
CoD Cortical Density
CoSA Cortical Sub Area
CoSD Cortical Sub Density
CT Cortical Thickness
CTX C-telopeptide of collagen
CV Coefficient of variation
CYP241 Cytochrome P450, family 24, subfamily A, polypeptide 1
CYP27B1 Cytochrome P450, family 27, subfamily B, polypeptide 1
DAYDYS Daytime dysfunction (PSQI subscale)
DEQAS Vitamin D External Quality Assessment Scheme
D-FINES Diet, Food Intake, Nutrition and Exposure to Sunlight in Southern England
DISTB Sleep disturbances (PSQI subscale)
DURAT Sleep duration (PSQI subscale)
DXA Dual x-ray absorptiometry
EC Endosteal Circumference
EPIC European Prospective Investigation into Cancer and Nutrition
FLx Fracture Load with respect to x axis
Fly Fracture Load with respect to y axis
GMT Greenwich Mean Time
HSE Habitual Sleep Efficiency (PSQI subscale)
HR-pQCT High Resolution peripheral Quantitative Computed Tomography
HPLC High Performance Liquid Chromatography
HRT Hormone Replacement Therapy
IDS Immunodiagnostic Systems Ltd
IMD Index of Multiple Deprivation
IU International Units
IS Inter daily stability
IV Intra daily variability
L5 Least 5 hours of activity
L5 Onset Time of onset of least 5 hours of activity
LATEN Sleep latency (PSQI subscale)
LC-MS Liquid chromatography–mass spectrometry
MED Minimal Erythemal Dose
MEDS Sleep medication (PSQI subscale)
MSFsc Mid-sleep point on free days corrected for sleep loss on workdays
M10 10 hours of most activity
M10 Onset Time of onset of 10 hours of most activity
NDNS National Diet and Nutrition Survey (UK)
NHANES National Health and Nutrition Examination Survey (USA)
NREM Non Rapid Eye Movement
NPCRA Non Parametric Circadian Rhythm Analysis
PC Periosteal Circumference
PER 3 Period circadian protein homolog 3
POST ASIAN Postmenopausal Asian
POST CAUC Postmenopausal Caucasian
PRE ASIAN Premenopausal Asian
PRE CAUC Premenopausal Caucasian
PSQI Pittsburgh Sleep Quality Index
PTH Parathyroid Hormone
pQCT peripheral Quantitative Computer Tomography
QUS Qualitative Ultrasound
PVN Paraventricular Nucleus
ROR Retinoic acid-related orphan receptor
RXR Retinoic acid receptor
SACN Scientific Advisory Committee on Nutrition
SAD Seasonal Affective Disorder
SCN Superchaismatic Nuclei
sCTX serum C-telopeptide of collagen
SED Standard Erythemal Dose
SES Socio-economic status
SJL Social jetlag
SLE Systemic Lupus Erythematosus
SLPQUAL Sleep quality (PSQI subscale)
SON Supraoptic nucleus
sPTH serum Parathyroid Hormone
SSI Strength-Strain Index
SSIp Polar Strength-Strain Index
SSIx Strength Strain index with respect to x axis
SSIy Strength Strain index with respect to y axis
SD Standard Deviation
ToA Total Area
ToD Total Density
Trab A Trabecular Area
Trab D Trabecular Density
QUS Quantitative Ultrasound
uNTX urinary N-Telopeptide of Collagen
UVB UltraViolet B irradiation
vBMD volumetric Bone Mineral Density
VDR Vitamin D receptor
VOS Velocity of Sound

Important note: In this Thesis the term ‘South Asian’ or ‘Asian’ will be used to denote individuals whose ethnic origin is that of the South Asian sub-continent (Bangladesh, India, Pakistan) as well as those individuals who have South Asian ancestry but originate from other countries (e.g. East Africa). The term ‘Caucasian’ will be used throughout as shorthand for ‘White Caucasian’
CHAPTER 1- Introduction
1.1 General

In recent years the interest in vitamin D has been phenomenal. A search of the PubMed database (Pubmed.gov) by the present author suggests that of the 60 000 published peer reviewed papers on the subject (since the first published paper on vitamin D in 1922), around two thirds have been published in the last twenty years. Much of the exponential growth in the number of vitamin D related publications is likely due to the increasing recognition that vitamin D deficiency is associated with a range of adverse health outcomes. These health outcomes include osteoporosis, heart disease, cancer, diabetes mellitus and autoimmune disease (Holick 2007).

Most populations worldwide are at risk of vitamin D deficiency, with some groups at especially high risk (e.g. persons from the Middle East and Asia) (van Schoor and Lips 2011). There is a lack of information about vitamin D status and its health effects in some of these high risk populations, such as South Asians. Moreover, there is a lack of information about the social influences on vitamin D status, via sunlight exposure. This Thesis uses a multidisciplinary approach to assess this problem, combining the disciplines of Nutrition, Sociology and Chronobiology to examine the biological and social influences on low vitamin D levels and low sunlight exposure in UK dwelling South Asian and Caucasian women. It also examines the likely health effects of this, with reference to two health conditions; musculoskeletal health and sleep quality. This information will be useful in guiding the production of effective strategies to improve vitamin D status in UK dwelling South Asian women, in order to improve health.

1.2 Vitamin D and musculoskeletal health

1.2.1 Vitamin D and health

Vitamin D is a fat soluble secosteroid. It has two forms, vitamin D2 (ergocalciferol) and D3 (cholecalciferol) (Figure 1.1). The name vitamin D is a misnomer as it is actually a prohormone, not a true vitamin (‘vital amine’). The main source is not dietary, but via synthesis in the skin’s epidermis. Vitamin D is produced from the UVB (Ultra Violet B) irradiation of 7-dehydrocholesterol in the skin. The main ‘classical’ role of vitamin D is to enhance the absorption of dietary calcium from the intestine, which has the crucial function of maintaining calcium homeostasis and ensuring musculoskeletal viability. More recently it has also been associated with ‘non classical’ roles. These include the regulation of immune function, hormone secretion, cell proliferation and cell differentiation (Bikle 2009).
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Traditionally, vitamin D deficiency has been associated with the childhood bone disease rickets. It has now been linked with a wide range of other diseases such as heart disease, osteoporosis, diabetes, multiple sclerosis, infectious diseases and dementia (Holick 2007). There is a high prevalence of vitamin D deficiency worldwide (van Schoor and Lips 2011) and vitamin D deficiency is increasingly being recognised as a significant and costly health problem (Grant et al. 2009). Grant et al. (2009) estimated that if average vitamin D levels, as measured by 25-hydroxyvitamin D (25(OH)D) in Western Europe could be raised to 40nmol/l then 187,000 million Euros per year could be saved in direct and indirect costs to society (Grant et al. 2009). More recently, another study estimated that all-cause mortality, worldwide, could be reduced from 7.6% to 17.3% if 25(OH)D concentrations were raised, on average, from 54nmol/l to 110nmol/l (Grant 2011). As a result, it is clear that vitamin D deficiency has potentially enormous social and economic consequences.

1.2.2 Vitamin D, ethnicity and musculoskeletal health

Vitamin D deficiency is associated with chronic musculoskeletal disease, which carries a high morbidity and mortality. This is due to the fact that vitamin D deficiency causes poor bone mineralisation, which leads to lower bone density. Also, vitamin D deficiency causes abnormalities in muscle composition and structure, which lead to poorer muscle strength (Ceglia 2008). Vitamin D supplementation has been found to improve balance and muscle strength (Muir and Montero-Odasso 2011) and reduce falls risk in the elderly (Kalyani et al. 2010). Vitamin D deficiency also causes chronic musculoskeletal pain due to swelling of the under mineralised bone tissue. Chronic pain is known to be associated with other health problems, such as poor ability to undertake daily activities (Houston et al. 2013) and poor sleep (Dikeos and Georgantopoulos 2011). Therefore, chronic pain due to vitamin D deficiency is likely to increase the risk of these other problems. A
concurrent low overall sunlight exposure may also cause further problems for sleep health, (this will be elucidated further in Section 1.3).

There has been some research into ethnic differences in musculoskeletal problems. For example, it is known that there is an increased prevalence of chronic musculoskeletal problems in older, UK dwelling South Asian and Black women as compared to same age Caucasian women (Allison et al. 2002). This is alongside an increased risk of other chronic illness such as type 2 diabetes mellitus, obesity and high blood pressure in South Asians (Misra and Khurana 2011). South Asian women are also known to have poorer vitamin D status than Caucasian women (Darling et al. 2013; Patel et al. 2012) or Black women (Patel et al. 2012). The extent to which vitamin D deficiency is associated with this increased risk of musculoskeletal problems in South Asians is unclear, due to confounding ethnic differences in body composition, physical activity, physiology and genetics.

1.3 Light exposure and sleep

1.3.1 Light exposure and circadian rhythms

Light exposure is important in entraining the body’s circadian rhythms to the 24 hour day, helping to establish healthy rest-wake cycles. The ability to adapt to, and alter, behaviour appropriately to changes in light levels over the course of the day and the year is crucial, with the body using this information to assess time of day and season. This ensures animals are active or asleep at the best times of day for their survival.

The mechanisms the body uses to interpret these crucial changes in light have been well elucidated. In mammals, there are two bundles of cells in the brain called the Suprachiasmatic Nuclei (SCN) which contain the central circadian ‘pacemaker’. The SCN receives light impulses from the retina of the eye, via the retino-hypothalamic tract. By this mechanism, light is able to entrain the central circadian pacemaker to the 24 hour day. The central pacemaker then regulates peripheral body clocks or ‘peripheral pacemakers’ contained in the other organ systems of the body. The importance of light for circadian entrainment cannot be underestimated. Light exposure is the primary regulator (‘zeitgeber’) entraining the SCN and rest-wake cycles in humans. Without light, the circadian system starts to ‘free run’. This means it becomes out of synchrony with the external environment, and can lead to sleeping and being awake at the wrong times of day (e.g. being awake at night and asleep in the day, as seen in some blind individuals (Sack et al. 1992)).
Although light is the primary influence on the circadian clock, other factors may also have an influence. Research suggests that physical activity (reviewed by Hughes and Piggins 2012) and social factors (Wever 1980) might influence circadian rest-wake cycles. However, the importance of these non-light (‘non-photic’) factors in entrainment of the clock is not well elucidated (reviewed by Mistlberger and Skene 2005) and are likely to be much less important for entrainment than light.

Brighter light has been found to produce a higher degree of entrainment of circadian rhythms than less bright light (Goff and Finger 1966). However, it is not just the amount of light that the eye receives that is important, but also the time of day of exposure, for two main reasons. First, morning light contains a higher density of the shorter wavelengths (i.e. ‘blue’ light) which the circadian system is more sensitive to than other wavelengths (Lockley et al. 2003) and thus gives the most effective circadian entrainment. Blue wavelength light is at its maximum in the early morning, tailing off in the early afternoon to a minimum in the evening (Thorne et al. 2009). Second, the timing of light exposure has an influence on entrainment and the timing of the circadian system (Khalsa et al. 2003). Most humans have an endogenous tau (phase length of the circadian cycle) of slightly longer than 24 hours. Consequently, in order to keep a circadian phase of 24 hours, to keep a regular sleep-wake cycle with the environment, the circadian cycle needs to be shortened (i.e. advanced slightly each day). Exposure to light in the morning causes a phase advance in the circadian cycle, whereas afternoon light causes a phase delay in the cycle (Figure 1.2).

![Phase Advance and Delay](source: adapted from (Barion and Zee 2007))

**Figure 1.2 Phase advance and delay due to light exposure at different times of day.**

A one hour pulse of light has been shown to cause a clear advance or delay in peak melatonin concentrations (a marker of circadian phase) throughout the day (St Hilaire et al. 2012) (Figure 1.3).
source: adapted from (St Hilaire et al. 2012)

Figure 1.3: Phase changes due to 1h bright light pulse at 3-4am (night) (Panels A and B) and at 7-8am (morning) (Panels C and D), using melatonin as a marker of circadian phase

This means that most people require some early morning light exposure to get their circadian system entrained to exactly a 24 hour day. If they do not get this light, they will ‘free run’ slightly, so they will go to bed later and wake up slightly later each day, which is disruptive to their sleep-wake cycles. There are a few individuals who have a tau shorter than 24 hours. These people will need to be exposed to light in the afternoon, to cause a circadian phase delay which will lengthen their circadian cycle to 24 hours. Light at night for all individuals will also cause phase delay. Overall, adequate light exposure at the correct time or day is crucial for entrainment of the circadian system, and is likely to be important for ensuring good sleep quality.

1.3.2 Mechanistic associations between the circadian system and sleep problems

The two process model of sleep was proposed in the early 1980s (Borbely 1982) This model explains sleep wake cycles via two drives, the homeostatic drive (Process ‘S’, time since wake) and the circadian drive (Process C) (Figure 1.4).
Throughout the daytime period the homeostatic drive builds up increasing sleep pressure which aids the onset of sleep, and the maintenance of sleep in the early sleep stages. However, the power of this drive fades after a few hours of sleep. The circadian process is linked to the timing of the melatonin system, with a rise in the evening leading to increased sleep propensity aiding sleep initiation, maximum levels in plasma 02:00 h to 04:00 h. It is important that the circadian system is correctly entrained to the 24h period, so that it is able to aid sleep maintenance once the sleep debt (homeostatic component) has been paid back after the first few hours of sleep. Overall, light exposure is important as it enables individuals to achieve a good robust circadian rhythm with a high amplitude (Jewett et al. 1994), which helps to get a clear difference between rest and activity, night and day.

1.3.3 Prevalence of sleep problems

Sleep problems are common and can range from mild sleep disruptions to diagnosed insomnia. In the UK population, the prevalence of acute insomnia is high, at 30-35% (Ellis et al. 2012). In addition to this, there are likely to be many more people with less severe sleep problems that still exact a clear morbidity to the individual and to society.

The prevalence of sleep related problems varies by gender and ethnic group. Some US studies have found that men from ethnic minority groups (Hispanic, Asian, Native American and African American) have the poorest sleep (Jean-Louis et al. 2000) and that ethnic minority women (Hispanic, Native American and African American) have poorer sleep than Caucasian women (Kripke et al. 2004). Whilst there may be socio-economic or genetic reasons for some of these differences in sleep quality, differences in light exposure may play some role in explaining these differences (Kripke et al. 2004). Returning to the discussion of South Asian and Caucasian women, recent work has confirmed that South Asian women have a lower UVB exposure than Caucasian women, (Macdonald et al. 2011; Darling et al. 2013) so they may also have a lower light exposure. The
generalizability of these findings across the wider population of South Asian women and the possible impact of a reduced light exposure on rest-wake cycles and sleep quality remains to be seen.

1.4 Vitamin D, musculoskeletal health, light and sleep

1.4.1 Epidemiology and the determinants of vitamin D status

1.4.1.1 Vitamin D in the UK

There has been a large amount of empirical research in recent years into vitamin D status and the prevalence of vitamin D deficiency and insufficiency in the Western world. This research suggests the problem of vitamin D deficiency has reached potentially epidemic proportions. For example, UK studies show a high prevalence of vitamin D deficiency and insufficiency in the majority Caucasian population (Atherton et al. 2009; Tolppanen et al. 2012). Data from the 1965 Birth Cohort (Hypponen and Power 2007) suggested that 15.5% of middle aged British Caucasians were deficient (<25nmol/l), 46.6% insufficient (<50nmol/l) and 87.1% sub-optimal (<70nmol/l) for 25(OH)D in winter and spring (Hypponen and Power 2007). Studies in Europe and the USA also confirm similar findings for Caucasian groups (Turer et al. 2013; Cutillas-Maro et al. 2012).

Vitamin D deficiency tends to be most common in darker skinned individuals, including both western dwelling South Asian populations and those dwelling in their country of origin (Sachan et al. 2005). Indeed, 50% of postmenopausal women in Southern India had 25(OH)D between 25 and 50nmol/l, with 18% having 25(OH)D over 50nmol/l, and 30% under 25nmol/l (Harinarayan 2005). Of concern, there is a high prevalence of vitamin D deficiency in immigrant and ethnic minority groups in Europe as shown by studies in Belgium (Moreno-Reyes et al. 2009), Holland (van der Meer et al. 2008), Norway (Henriksen et al. 1995) and the UK (Macdonald et al. 2011) (Darling et al. 2013). There have been similar findings in ethnic minority groups in the USA (McKinney et al. 2008), New Zealand (von Hurst et al. 2009) and Australia (Brock et al. 2004). UK dwelling South Asian populations have been found to have very low levels of 25(OH)D (Macdonald et al. 2011) (Darling et al. 2013) with 51.4% of older South Asian postmenopausal women in the South of England classified as deficient (<25nmol/l), and 88.6% as insufficient (<40nmol/l) in summer (Macdonald et al. 2011). Also, very high rates of insufficiency have been found in younger South Asian women living in the UK (Darling et al. 2013) and in Norway (Holvik et al. 2007).

Much research has, perhaps disproportionately, focused on describing this deficiency and debating a definition of optimal and sufficient status. Recent and growing attention has been paid to the factors
influencing vitamin D status, including the relative contributions of UVB exposure and dietary vitamin D intake. Research has also investigated the contributions of other environmental and lifestyle factors as well as physiological factors. It is necessary to understand these factors to recognize why certain ethnic groups are more prone to poor vitamin D status.

1.4.1.2 Production of vitamin D in the skin

The ability of the skin to produce vitamin D is dependent on the wavelength of UVB radiation being received from the sun. At latitudes far from the equator, the emitted UVB wavelength varies greatly at different times of the year, due to differing angles of the sun in relation to the earth. For that reason, such latitudes do not receive as much UVB of the correct wavelength for vitamin D production, for as much of the year, as do latitudes nearer the equator. At latitudes further north or south than around 30\(^0\) degrees latitude, no vitamin D is made from October to April. As can be seen in Figure 1.5, only some parts of Africa, the Middle East, South Asia, South America and Australasia have UVB wavelengths of sufficient intensity to produce Vitamin D all year around.

![Figure 1.5: Sufficiency of UVB wavelength by geographical location](source: (Tavera-Mendoza and White 2007) (pg. 65))

For South Asians, migration to the UK means having moved from an area of the world where vitamin D can be produced all year around, (even in winter) and the UVB irradiation is intense, to an area of the world where vitamin D can only be made for part of the year and the UVB irradiation is less intense. This is likely to have detrimental implications for their vitamin D status as a population group which is compounded by other ethnicity-related factors influencing skin production of vitamin D.
1.4.1.3 Vitamin D production and ethnicity

The two main factors influencing the production of vitamin D in the skin are amount of skin exposed to the sun, and skin absorption of UVB rays. Most South Asian women tend to have a more covered dress style than Caucasian women, for religious and cultural reasons. This dress style may include covering the head, as well as all of the arms and legs, in all seasons, including summer. There are lay reports that sunscreen is also commonly used on the face and hands, although this has not been officially documented in the literature to the author’s knowledge. The problem for vitamin D status is that a covered dress style (and possible usage of sunscreen) block UVB rays reaching the skin, thus preventing vitamin D production (Matsuoka et al. 1987) (Matsuoka et al. 1992). Veiling, as practised in South Asian and Middle Eastern populations, has been found to be detrimental to vitamin D status in some studies (Gannage-Yared et al. 2000) but not others (Islam et al. 2006; Mishal 2001). In addition, skin colour is known to influence the ability to produce vitamin D. Darker skin contains more melanin, which filters out UVB rays. Some research has shown that 25(OH)D production is slower in darker skin at a set UVB exposure (Clemens et al. 1982). This suggests that South Asians need to be exposed longer to the UVB radiation to produce the equivalent amount of 25(OH)D to Caucasians.

1.4.1.4 Dietary vitamin D

Dietary intake (vitamin D₂ and D₃) is known to be the second major source of vitamin D. It can be obtained from food or from dietary supplements, but few foods contain significant amounts of vitamin D. The most concentrated foods are oily fish, eggs, cereals and fortified spreads, but these foods still only provide the body with small amounts of vitamin D. A recent study found a consumption of only 2.0 to 2.6 micrograms/d in a non-supplemented diet, in UK South Asian and Caucasian women (Darling et al. 2013). Some studies suggest diet is a significant contributor to vitamin D status (Mithal et al. 2009) but others do not (Darling et al. 2013). This is likely due to the low amounts of vitamin D in most diets. In contrast, dietary supplements, providing vitamin D at higher doses (10 micrograms or more per day) are known to be effective in improving vitamin D status. Each increase in dose of vitamin D₃ by 2.5micrograms (100IU) increases serum 25(OH)D by 2.5nmol/l (Heaney 2008). Therefore, a 10 microgram per day (400IU) supplement would raise 25(OH)D by 10nmol/l.

Although diet is only a minor source of vitamin D for most population groups, it may be a more important contributor for groups who have little sunlight exposure. An examination of diet may help explain why South Asian women have poorer vitamin D status than Caucasians. Describing general dietary patterns in South Asian groups is very difficult, as South Asian diets are very heterogeneous,
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varying by region of migratory origin as well as by religious practices and degree of westernisation. Despite this, it is known that older South Asians tend to adhere to a traditional diet that resembles closely that of the country of origin. This is in contrast with younger South Asians, who tend to combine aspects of a traditional diet with that of the western diet. Region of origin and religion is also very important in dietary choice. For example, Hindus are usually vegetarian whereas Muslims are usually not. Some Muslim women do not eat large amounts of fish (e.g. Pakistani), but some do (e.g. Bangladeshi).

Due to oily fish being the most concentrated food source of vitamin D, whether fish is consumed regularly, or at all, is likely to be one of the most important dietary influences on vitamin D status. It is likely that the lowest dietary intake of vitamin D is seen in South Asians with a strict vegetarian or even vegan diet, with no meat or fish and little or no intake of eggs. Research on Hindu South Asians living in the UK found poorer vitamin D status in strict vegetarian Hindus than in non-vegetarian Muslims (Finch et al. 1992) but these results are complicated to interpret. It must be borne in mind that stricter diets may be associated with a more traditional lifestyle which itself includes more practices which might limit sun exposure (e.g. indoor activities, covered dress style). Even ‘good’ dietary intakes of vitamin D (e.g. 5 micrograms per day) may not translate to clinically relevant improvements in vitamin D status. In the absence of a reasonable sun exposure, much larger doses of vitamin D would be required to raise 25(OH)D concentration to 50nmol/l, the most recent definition of ‘sufficient’ status advised by the US Institute of Medicine (IOM 2011). This is likely to be achievable only with increased sun exposure or vitamin D supplementation, although uptake of the latter remains highly variable within and between population groups. The amount of vitamin D in over-the-counter supplements in the UK has traditionally been low (e.g. 200-400IU/d), although higher doses (e.g. 1000IU/d) are now being sold (Gillie 2010). Public health schemes, such as Healthy Start in the UK, have been criticised for failing to promote uptake of vitamin D containing supplements (Gillie 2010). A recent study found that only 52% of health visitors were aware of recommendations for vitamin D supplements for UK infants (Locyer et al. 2011), with few promoting awareness of the free Healthy Start vitamins. This is despite the Department of Health (DOH 1991) recommendation of a supplement of 10 micrograms per day for all persons at high risk of vitamin D deficiency. This includes individuals with low sun exposure (e.g. housebound elderly), those who are pregnant, and those with darker skin pigmentation (DOH 1991). However, this recommendation has not led to the correct supplementation of most at risk individuals. In young South Asian women, vitamin D supplement usage has been found to be almost non-existent (Darling et al. 2013). There are no data on vitamin D supplement use in older South Asian women, despite the fact that they may be at greater risk of vitamin D deficiency than younger women due to the additional detrimental effect of ageing on vitamin D status. Ageing may detriment vitamin D status by causing poorer mobility, which leads to reduced outdoor exposure (Janssen et al. 2002). Older people may have a higher requirement for vitamin D due to poorer renal activation of 25(OH)D to
the active hormone 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) (Vieth et al. 2003). This means that the expected low consumption of vitamin D supplements in older South Asians living in the UK is of particular concern, especially if sun exposure is low and there is very little vitamin D in the diet.

1.4.1.5 Other physiological factors affecting vitamin D status

Other physiological variables are also likely to be of importance in explaining the high prevalence of vitamin D deficiency in UK South Asian populations. First, South Asian populations have a high prevalence of obesity (reviewed by Misra and Khurana 2011). This is a problem as body fat may sequester vitamin D, which may lead to poorer vitamin D status. Lower 25(OH)D with increasing obesity has been found in many studies (Ernst et al. 2009; Beydoun et al. 2010), whilst persons with a higher fat mass have a smaller response of 25(OH)D to vitamin D supplementation (Gallagher et al. 2012). Second, there is emerging evidence that genetic polymorphisms in the vitamin D receptor (VDR) or enzymes involved in vitamin D metabolism may also be an important influence on vitamin D status (McGrath et al. 2010). In fact, a recent paper by Hunter et al. (2001) found 43% of the variance in 25(OH)D and 65% of 1,25(OH)$_2$D to be explained by genetic factors (Hunter et al. 2001). Also, in the SUNLIGHT consortium genome-wide association study, Wang et al (2010) identified 3 genetic loci associated with vitamin D insufficiency. These loci included DHCR7, CYP2R1 and GC (Wang, 2010). DHCR7 is the enzyme responsible for breaking down the vitamin D precursor 7-dehydrocholesterol back to cholesterol. The biological role of CYP2R1 is not clear, but may be one of the enzymes which hydroxylase 25(OH)D to 1,25(OH)$_2$D (i.e. has 25-hydroxylase activity). Finally, GC is the vitamin D binding protein, which carries vitamin D to and from the body tissues. It is not clear as to whether there are ethnic differences in these loci, as well as other genes relevant to vitamin D metabolism. However, it is likely that different ethnic groups possess differences in these polymorphisms which potentially contribute to explaining some of the ethnic differences in 25(OH)D. Which ethnic differences in genetics affect vitamin D status are not clear, and in particular those which are important in explaining differences in vitamin D metabolism are unknown. Few studies have assessed genetics and vitamin D metabolism in South Asian groups, with one of the few studies that have investigated this area showing no association between vitamin D binding protein genotype and 25(OH)D in South Asians (Gozdzik et al. 2011). Overall, more research is required to elucidate the genetic influences on vitamin D status, and any variation in these between and within ethnic groups.
1.4.2 Vitamin D status trends

1.4.2.1 Within year change: Ethnic differences

Vitamin D levels are not static. Concentrations of the serum metabolite, 25(OH)D, vary over the period of a year. There is a known peak in summer/autumn and a nadir in winter. This is due to the fact that in the UK, no vitamin D can be produced in the skin from October to April due to insufficient UVB wavelength for vitamin D production, so that the body stores of vitamin D must be used as a source. Hence, in the UK, and at equivalent or higher latitudes, vitamin D status commonly varies throughout the year.

Recent work on circannual (i.e. ‘yearly’) changes in 25(OH)D in the UK has shown seasonal variation in 25(OH)D in older Caucasian women, with older South Asian women showing only a small response of 25(OH)D to seasonal change (Macdonald et al. 2011). Similarly, vitamin D status has been shown to fluctuate less by season in UK dwelling premenopausal South Asian women, than in same age Caucasian women (Darling et al. 2013).

As discussed earlier, it is known that low levels of vitamin D are detrimental to a wide range of health outcomes. Therefore it can be assumed that populations with low vitamin D levels, such as the South Asian population, are at a health disadvantage. However, there has been a recent suggestion in the literature that higher seasonal variation in vitamin D status may also be detrimental for some health outcomes (Vieth 2004). This may suggest a hidden health advantage in the minimal change in 25(OH)D by season in UK dwelling South Asian women.

1.4.2.2 Within year change: Seasonal fluctuation of 25(OH)D status and bone health

It has been shown that globally, 25(OH)D concentration decreases with increasing geographical latitude (Hagenau et al. 2009). For example, due to their high Northern latitude, the prevalence of vitamin D deficiency has been shown to be high in individuals living in Europe (Lips 2007) and Canada. The seasonal variability in UVB radiation at higher latitudes also leads to noticeable seasonal variation in serum 25(OH)D concentration (Mithal et al. 2009; Oliveri et al. 1993). These seasonal differences are large compared to those of rural dwelling humans living closer to the equator (Prentice et al. 1997)

The situation is further complicated by the inter-individual variation in seasonal serum 25(OH)D within populations (Darling et al. 2013; Macdonald et al. 2006). Some individuals show far larger changes in serum 25(OH)D concentration than others across seasons. The reasons for these
individual differences are not clear, but differences in sun exposure behaviour (Darling et al. 2013; Macdonald et al. 2006) ethnicity (Darling et al. 2013; Macdonald et al. 2006; Finch et al. 1992) and clothing style (Darling et al. 2013) may be responsible. Recent work in premenopausal UK women has shown that intra individual (e.g. seasonal) factors are as important as inter-individual factors in determining vitamin D status (Darling et al. 2013). The few studies that have investigated seasonal changes in 25(OH)D concentration have found that South Asians (Darling et al. 2013; Macdonald et al. 2006; Finch et al. 1992) and older people from all ethnic groups (Lester et al. 1977; Lagunova et al. 2009) show less pronounced seasonal variation in their 25(OH)D concentration than other population sub-groups, including younger adults and Caucasians. This is in addition to having overall lower 25(OH)D concentrations than other population sub-groups (Lowe et al. 2010).

Large seasonal changes in 25(OH)D are of interest as it has been hypothesised that they may have consequences for the activity of the hydroxylase enzymes that control vitamin D metabolism. These enzymes include 1-hydroxylase (cytochrome P450 27B1; CYP 27B1), which catalyses the conversion of the substrate 25(OH)D to 1,25(OH)2D. Also, 24-hydroxylase (cytochrome P540 24A1; CYP24A1) catalyses 25(OH)D to 24,25dihydroxyvitamin D (24,25(OH)2D) as well as catalysing 1,25(OH)2D to 1,24,25trihydroxyvitamin D (1,24,25(OH)3D) (see Figure 1.6).

![Diagram of vitamin D hydroxylase enzymes](source: Bailey et al, 2013)

Figure 1.6: The actions of vitamin D hydroxylase enzymes

The activity of the 1-hydroxylase enzyme is readily affected by changes in the concentration of its 25(OH)D substrate. This is because, unlike many other enzymes, it is working well below its Michaelis-Menten constant (Km) at physiological concentrations of 25(OH)D.

As a result, large seasonal fluctuations in 25(OH)D substrate could cause large changes in the activity of the 1-hydroxylase enzyme (Vieth 2009). Theoretically, a long term decline in levels over the course of the year will not allow the desired level of 1,25(OH)2D to be achieved until the decline
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finishes (Vieth 2009). This suggests that individuals with large seasonal change in 25(OH)D concentration may have sub-optimal 1,25(OH)₂D concentration for much of the year. In support of this, a recent study assessing seasonal changes in serum 25(OH)D and 1,25(OH)₂D concentrations in a Norwegian population (62°N) suggests that, at least in some individuals, circulating 1,25(OH)₂D concentration does fluctuate by season (Christensen et al. 2010) and mirrors fluctuation in 25(OH)D concentration (Lagunova et al. 2009).

There is no evidence to date as to whether regular large seasonal changes in 25(OH)D concentration have any effect on health. There has been some suggestion of potential harm, based on findings of increased risk of prostate and pancreatic cancers (Tuohimaa et al. 2004; Brock et al. 2010) and findings of increased mortality (Michaelsson et al. 2011) in individuals with high vitamin D status. It has been proposed by Vieth (2004) that these detrimental effects could be due to seasonal changes in 25(OH)D rather than high 25(OH)D per se. This is because individuals with high serum 25(OH)D concentrations tend to be those who show the most seasonal change in 25(OH)D (Vieth 2004). Therefore, they are potentially susceptible to the detrimental perturbations in the activity of the hydroxylase enzymes described above. This intriguing hypothesis proposed by Vieth (2004) to explain the increased cancer risk begs the question as to whether seasonal fluctuation or ‘cycling’ of 25(OH)D could also be detrimental to other aspects of health. A recent study suggested flares in the autoimmune disease Systemic Lupus Erythematosis (SLE) may be precipitated by large changes in vitamin D status (Birmingham et al. 2012). It is unknown whether these large seasonal fluctuations in 25(OH)D may also have an impact on bone health.

The paracrine and autocrine effects of 1,25(OH)₂D, produced locally in bone cells by 1-hydroxylase from 25(OH)D, have been recently elucidated (van Driel et al. 2006; Atkins et al. 2007). Not enough is currently known about hydroxylase enzyme activity in bone cells to assess whether fluctuations in the 25(OH)D substrate would have any detriment on their ability to produce 1,25(OH)₂D in the correct quantities. Indeed, in bone cells, 1-hydroxylase and 24-hydroxylase have been found to be positively coupled, unlike in kidney cells where they are inversely coupled (Anderson et al. 2005). This paracrine and autocrine vitamin D activity is important for many bone cell processes, including mineralisation (Need et al. 2007) and regulating osteoclast differentiation and activity (Anderson et al. 2008). It is unknown whether seasonal fluctuations in 25(OH)D concentration could cause adverse perturbations in this regulation, and thus be detrimental to bone health. Some studies show that bone markers show seasonal variation (Woitge et al. 1998) but other studies do not (Blumsohn et al. 2003). Even if bone markers do show seasonal variation, it could possibly be explained by other processes (e.g. circannual rhythms) rather than changes in vitamin D metabolism.
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It is unknown whether people showing a larger change in 25(OH)D over the course of a year show increased bone resorption in comparison to those with a smaller change in 25(OH)D. If so, this would imply that the smaller the change in 25(OH)D, the smaller the bone resorption. It would also suggest that South Asians (who are stable in 25(OH)D) may have lower bone resorption than Caucasians (who are less stable in 25(OH)D). This is likely to be a complicated relationship, whereby those with higher 25(OH)D show more seasonal change than those with lower 25(OH)D, meaning there is an interaction between seasonal change in 25(OH)D and average 25(OH)D concentration.

It would be interesting in the context of this Thesis, and for understanding the musculoskeletal effects of Caucasian and South Asian vitamin D status, to see whether the stable, but lower vitamin D levels in South Asians are beneficial to bone health, as compared to the higher, but strongly fluctuating vitamin D levels likely to be seen in Caucasians. Of course, it is not only status fluctuations over the course of the year that are important for health, change in average concentration of 25(OH)D over a period of years is also likely to be important.

1.4.2.3 Between year change: Ethnicity and vitamin D status

Data on longer term changes in vitamin D status over the period of several years, or decades are sparse in the literature. The largest national study undertaken to date looking at longitudinal change in 25(OH)D over a period of years was from the N-HANES survey (USA, (Ginde, Liu, and Camargo 2009). This survey showed a significant decline in 25(OH)D from 75nmol/l (1998-1994) to 60nmol/l (2001-2004) for non-adjusted data (Ginde, Liu, and Camargo 2009), whereas age adjusted values showed a decline of 5-20nmol/l (Looker et al. 2008). When the N-HANES data was assessed by gender, males showed a decrease in age adjusted serum 25(OH)D by 5-9nmol (from the 1988-1994 to the 2000-2004 survey), whereas there was no change for females (Looker et al. 2008). In contrast, a large Canadian survey (1995-2007) has found a recent increase in 25(OH)D concentration, which the authors speculate is likely to be due to increased use of vitamin D supplements in the Canadian population (Berger et al. 2012).

In terms of smaller national studies, a recent Norwegian study by Jorde et al. (2010) compared banked blood samples (from 1994 and 2008) for 25(OH)D concentration, and found little change in mean 25(OH)D (53.7nmol/l and 55.3 nmol/l in 1994 and 2008 respectively). There was little change within subjects, with most people, especially those with very high or low 25(OH)D, staying within their quintile (Jorde et al. 2010). The National Diet and Nutrition Survey (NDNS) 2008/9 to 2010/11 found 17% of males and 19% of females (19-64 years) to be below 25nmol/l in the UK (DOH 2011). This was slightly higher than the 14% of all 19-64 year old men and 15% of 19-64 year old women.
in the previous survey in 2004 (Ruston 2004). Also Hirani et al. (2010) found no improvement in 25(OH)D status in older people between the 2000 and 2005 Health Surveys for England (Hirani et al. 2010). Interestingly, this change could not be explained by an ageing population as age was controlled for in the analysis, suggesting a real decline in vitamin D in older people in England, over a five year period.

Overall, the small number of studies assessing changes in 25(OH)D over a period of years tend to suggest a levelling off, or a slight reduction in 25(OH)D over the past decade or so. As would be expected, these results differ depending on country and population sub-group. Also, there is the problem that national surveys do not follow up exactly the same people over time, although the sampling in large studies is assumed to be reasonably representative of the population at both time points. Of course, it is important to assess the predictors of change in 25(OH)D, as well as the amount of change. Longitudinal measurements, like those surveys described above, can highlight the important factors influencing change in 25(OH)D status. For example, the Norwegian study showed that change in Body Mass Index (BMI), change in supplement use (including cod liver oil) and change in physical activity were significant predictors of change in 25(OH)D (Jorde et al. 2010).

Overall, there is a clear lack of research into changes in vitamin D status over time, in all countries. There has also been little examination of different age or ethnic groups separately. There is no UK data, to the author’s knowledge, on changes in the vitamin D status of South Asians over a period of years. Also, there are few studies that follow up the same people, over a period of years. As can be seen above, there is also a lack of longer term work on predictors of 25(OH)D concentration over a period of years, in Caucasians as well as Asians. It would be useful to see whether the extent of between year change in 25(OH)D differs between South Asians and Caucasians, and what the predictors of long term change in 25(OH)D are in these ethnic groups.

1.4.3 Musculoskeletal health

1.4.3.1 Controversy surrounding 25(OH)D status and musculoskeletal health

Musculoskeletal pain is a frequently encountered, but possibly under-documented health problem. The exact prevalence of vitamin D induced bone and muscle pain and any variation by ethnic group, is relatively unknown. There is growing evidence that musculoskeletal pain is a common problem in dark skinned populations in the UK. For example, in an analysis of UK survey data, Macfarlane et al (2005) found an increased age and sex adjusted odds ratio of musculoskeletal pain in South Asians (OR=1.6) compared to White Europeans (OR=1) (Macfarlane et al. 2005). Also, another study
found that musculoskeletal pain was more common in UK ethnic minority groups than in Caucasians (Allison et al. 2002).

There has been a call for more attention to be paid to the problem of musculoskeletal pain in ethnic minority groups (Nellen et al. 1996; Sievenpiper et al. 2008; de Torrente de la Jara et al. 2006). There have been few large scale research studies on the subject, with most studies conducted using small groups of pain clinic patients, or reporting single patient case studies. Overall, there is a suggestion that musculoskeletal pain is seen disproportionately more often in the UK South Asian population, as compared with the Caucasian population. More, larger scale research is required to accurately assess the prevalence of the problem and its implications.

It is also unclear the extent to which this prevalence of musculoskeletal pain is associated with poor vitamin D status. As discussed previously, it is clear that darker skinned ethnic minority populations have an increased risk of both low 25(OH)D concentration, and probably also of musculoskeletal pain. The hypothesis that this pain is due to vitamin D deficiency is a plausible one. There is no clear consensus as to the cause of this pain. However, it is plausible that bone pain could be caused by osteomalacia, where pain occurs due to the swelling of under mineralised bone tissue. The bone is under mineralised due to inadequate calcification caused by vitamin D deficiency. This bone pain would be likely to lead to significant morbidity as chronic pain has been associated with functional impairment and low physical activity (Bjornsdottir, Jonsson, and Valdimarsdottir 2013). Also, vitamin D deficiency can also cause muscle weakness and pain. These muscular problems, due to vitamin D deficiency, are associated with difficulty performing everyday tasks in the elderly (Houston et al. 2011) as well as an increased risk of falls (Bischoff-Ferrari et al. 2009). Vitamin D deficiency pain commonly affects the large, weight bearing muscles of the body (e.g. back of thigh muscles). This muscle pain and weakness are caused by changes in the structure and composition of muscle fibres. Vitamin D deficiency causes these changes in muscle tissue due to both mechanical and genomic mechanisms. First, this can involve atrophy of the type II fibres, with increased fat deposits, inter fibrillary widening and fibrosis (reviewed by Ceglia 2009). These muscle type II fibres are those responsible for fast reactions to stimuli. Therefore, vitamin D deficiency may lead to a slower response time and poorer physical performance. Second, vitamin D deficiency causes genomic changes at the cellular level, with changes in muscle differentiation and proliferation (Annweiler, Montero-Odasso, et al. 2010) which is detrimental to muscle function.

Despite a clear biological mechanism linking vitamin D deficiency and musculoskeletal pain, the link between these types of symptoms and vitamin D status is still controversial. For example, in a USA study, 100% of pain clinic patients from African, Indian or Hispanic backgrounds had 25(OH)D concentrations below 50nmol/l, compared with 80% of those patients from white backgrounds (Plotnikoff and Quigley 2003). However, this study was criticised as it did not
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compare the clinic patients to the general population, so it is unclear as to whether these prevalence rates of vitamin D deficiency are higher than would be expected or not (Myers 2004). Knutsen et al (2010) also found that many clinic patients with musculoskeletal pain had insufficient vitamin D (<50nmol/l) (Knutsen et al. 2010). Again, it is difficult to establish from these studies whether vitamin D deficiency was more common in the clinic patients than the general population.

Looking at the wider picture, it is also unclear to what extent muscle strength and quality of life are associated with vitamin D status. In terms of subjective measures, a Turkish study found a significant negative association between vitamin D status and self-reported quality of life (Basaran et al. 2007). These quality of life reports measured self-rated estimates of pain, social and physical ability, mental function and general health (Basaran, Guzel et al. 2007). In terms of objective measures of muscle strength, an increased sit to stand test time was found in elderly persons with the lowest 25(OH)D status (Bischoff-Ferrari, Dietrich, et al. 2004). Similarly, Annweiler et al. (2010) found vitamin D deficiency to be negatively associated with walking speed, especially for fast walking tests (Annweiler, Schott, et al. 2010). Also in the elderly, poorer physical performance, as assessed by a variety of tests, was observed in individuals with 25(OH)D <50nmol/l compared with those with >50nmol/l (Wicherts et al. 2007). However, a systematic review of 16 studies in older people by Annweiler et al. (2009) found conflicting results between studies as to the relationship between vitamin D deficiency and physical performance, reflecting the contradictory nature of research in this field (Annweiler et al. 2009). The recent Institute of Medicine report (2010) concluded that there was not enough evidence for the association between vitamin D and muscle health to warrant muscle health data being used to inform the dietary requirement for vitamin D (IOM 2011). Also, it must be borne in mind that vitamin D deficiency could be a result of musculoskeletal problems, rather than the cause, and all the above studies were undertaken in elderly Caucasian individuals so may not be applicable to other population groups.

There has been much interest in differences in muscle function between population sub-groups. For instance, there is a known difference in muscle function by gender, with males usually scoring higher on muscle strength than females and younger adults scoring higher than older adults (Shaunak et al. 1987). There is a particularly notable decrease in muscle strength with ageing, which has been attributed to the increased sarcopenia and fat infiltration of muscle fibres, as well as a reduction in the amount of type II fibres (Lexell 1995). As a result, it is expected that of all adults, older females have the poorest muscle function.

There has also been some investigation as to whether there are ethnic differences in muscle strength. Some studies have found ethnic differences in leg muscle strength (Liang et al. 2007) quadriceps strength (Davis et al. 1999) grip strength (Haas et al. 2012) upper and lower extremity physical function (Araujo et al. 2010), walking speed (Davis et al. 1999) and ability to do chair stands (Davis
et al. 1999). However, some other studies have found no ethnic differences in muscle function. For example, some studies have found no ethnic difference in response to muscle strength training (Walts et al. 2008) grip strength (Araujo et al. 2010) or knee strength (Schiller et al. 2000; Ostchega et al. 2004). The above studies examined a wide range of ages as well as different ethnic groups, but mostly in the US (Caucasian, Black, Hispanic, Chinese and Japanese populations).

Overall, the literature on ethnicity and muscle function is very heterogeneous, comparing a wide variety of ethnic groups, of different genders and age, on a wide variety of different muscle function tasks. This makes it difficult to draw conclusions from such a diverse literature base. There is also a lack of studies assessing muscle function in South Asian groups. One exception was a study which found that Caucasians had higher quadriceps strength than South Asians (Shaunik et al. 1987). Another exception is a study that compared older Indian men (dwelling in India) with same-age American men, and found poorer grip strength in the Indian men (Albert et al. 2005). One limitation of this research is the cross country comparison, which is a major confounder in the analysis. It might be more valid to assess ethnic groups for muscle function within countries than between countries. This is because of the confounding differences seen between populations in terms of diet, lifestyle and environment. Indeed, the different diet and lifestyle of the Indian men to the American men could explain differences in muscle strength, which may disappear if the all the men had been raised in the same country. These two studies are the only published research in the literature base comparing Caucasian and South Asian muscle function.

It is important to assess whether this poorer muscle strength would also be apparent in South Asian women of older age, in comparison with their Caucasian counterparts. It is especially important to assess women of older age, due to their increased likelihood of having poor muscle function due to the known effects of ageing on muscle. Some research has found that South Asian women may have a higher prevalence of musculoskeletal pain than Caucasian women (Allison et al. 2002; Macfarlane et al. 2005). Despite this, there is no known literature, to the author’s knowledge which has assessed older South Asian women and muscle function.

It is known that vitamin D status is important for muscle function. Vitamin D has both genomic and non-genomic effects on muscle (reviewed by Ceglia 2008). Hence, many studies have assessed the relationship between 25(OH)D and muscle strength and function. A large number of studies in older Caucasians show a positive relationship between 25(OH)D and better overall physical performance (Houston et al. 2011; Houston et al. 2009) chair stands (Toffanello et al. 2012) faster walking time and grip strength (Toffanello et al. 2012) postural stability (Boersma et al. 2012) timed up and go (Muir and Montero-Odasso 2011) and measures of muscle strength (Houston et al. 2011). However, when BMI was controlled for in the analysis, one study found no relationship between 25(OH)D and
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Functional performance (McDermott et al. 2012) suggesting BMI might be an important confounder in the relationship between 25(OH)D and muscle function.

Little research has also assessed whether there are ethnic differences in the relationship between 25(OH)D and muscle function. One USA study assessed 25(OH)D and muscle strength in Black, White and Hispanic men, and found no association between 25(OH)D and muscle strength (Ceglia et al. 2011). A small number of studies have assessed the relationship between 25(OH)D and muscle strength in South Asian populations. The few studies that have been conducted are described here. First, one study found that maternal vitamin D status was associated with smaller arm muscle area in the child at 9-10 years, but not with grip strength (Krishnaveni et al. 2011). Second, another study found improvements in grip strength and the 6 minute walking test when young Indian men and women were supplemented with calcium and vitamin D (Gupta et al. 2010). However, a further study found no improvement in the muscle strength of young South Asian women when they were supplemented with vitamin D and calcium (Goswami et al. 2012). These studies did not compare the response of the South Asians with other ethnic groups, so it was not possible to assess ethnic differences. Also, in the latter two studies, calcium could also have been part of the mechanism for the muscle function improvements seen with improved vitamin D status.

The lower 25(OH)D concentrations in South Asian women, as compared with Caucasian women (Lowe et al. 2010; Darling et al. 2013) suggests that it is imperative than research assesses whether poorer vitamin D status is associated with worse muscle function in this population group. Also, it is known that South Asian women have a smaller lean body mass than Caucasian women even when body size is equal (Lear et al. 2009) This would suggest a potential detriment in muscle strength amongst South Asian women, as compared with Caucasian women.

1.4.3.2 Vitamin D, ethnicity and bone density

There is some concern that South Asian immigrants may have poorer bone health than the indigenous population. For example, a recent U.S. study found that South Asian women had a higher prevalence than Caucasian women of femoral neck osteoporosis (Khandewal, Chandra, and Lo 2012). South Asian women have also been found to have a higher incidence of wrist fracture than Chinese women, although South Asians did not differ from White Caucasian women on these measures (Khandewal, Chandra, and Lo 2012; Lofthus et al. 2008). To investigate these epidemiological findings of increased osteoporosis incidence, but similar fracture rates, it is important to examine the differences in bone geometry between South Asians and White Caucasians.
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Measures of bone geometry have been associated with prediction of fracture risk (Mackey et al. 2007; Bousson et al. 2011; Black et al. 2008) with much research assessing bone geometry in different population groups (Kim et al. 2011; Marshall et al. 2008; Nelson et al. 2004; Walker et al. 2011). There has been a lack of research into the bone geometry of South Asian women, with most studies only assessing areal bone mineral density (aBMD), using dual-x ray absorptiometry (DXA). These studies suggest a lower aBMD in South Asian compared with Caucasian women (Brooke-Wavell et al. 2008; Hamson et al. 2003), but aBMD measures do not assess true volumetric bone mineral density (vBMD), as they are influenced by actual bone size. The smaller bone size of South Asian populations has been found to explain the apparent lower aBMD in this ethnic group as compared with Caucasians (Roy et al. 2005; Cundy et al. 1995). Furthermore, research suggests that osteoporosis diagnosis varies when western dwelling individuals of South Asian descent are classified using aBMD according to Caucasian as opposed to South Asian reference ranges (Melamed et al. 2010), suggesting this underestimation of bone density may have clinical implications.

In order to understand if South Asians truly have a lower bone density than Caucasians, it is important that vBMD in South Asian, as compared with White Caucasian populations, are investigated. The known small bone size in South Asians suggests a biomechanical detriment that is likely to lead to increased risk of fracture. In order to know if South Asians are at increased fracture risk, it is important to assess their bone structure (e.g. cortical and trabecular density), as well as vBMD and size.

Peripheral Quantitative Computed Tomography (pQCT) measures bone size, vBMD and bone architecture. There have been very few studies assessing South Asian bone structure by this method. The study by Ward et al (2007) assessed the radius by pQCT in premenopausal UK South Asian women and showed a smaller cross sectional area, lower bone mineral content (BMC) and vBMD, smaller cortical thickness and cortical area in South Asians compared to Caucasians (Ward et al. 2007). Interestingly, ethnic differences in body size in this study did not explain these differences, and there was no difference in stress-strain index (SSI) by ethnicity despite the observed differences in bone geometry (Ward et al. 2007). The only other study to assess pQCT measures in adult South Asian populations found similar vBMD of the radius in young UK South Asian women compared to Caucasian women (Roy et al. 2005).

It must be emphasised that the above findings only relate to premenopausal women, and to the radius only. To the authors’ knowledge there has been no research using pQCT to examine bone geometry of either the radius or tibia in postmenopausal South Asian women, either dwelling in the UK or in South Asia. Therefore, bone geometry data are urgently needed in this group. The bone geometry in Chinese women has been recently examined in detail using High Resolution peripheral Quantitative
Computed Tomography (HR-pQCT), and the increased vBMD and cortical thickness found, as compared with Caucasians, may lead to increased bone strength, despite a smaller bone size (Walker et al. 2011; Walker et al. 2012; Walker et al. 2009; Liu et al. 2011). It would be clinically relevant to assess whether similar adaptations are present in South Asian women.

Although studies vary in their findings for specific sites and population groups, it is known that vitamin D deficiency has negative effects on bone health. There is some evidence that consumption of 800IU or more vitamin D per day reduces risk of fracture, (Bischoff-Ferrari et al. 2005) as well as falls (Bischoff-Ferrari, Dawson-Hughes, et al. 2004). However, it must be borne in mind that effects of vitamin D on bone may be confounded by the effects of exercise, disuse and ageing. Those individuals with the lowest vitamin D may also do little exercise, be in poorer health and of older age, all of which are known to have adverse effects on bone architecture. Higher 25(OH)D has been found to be associated with increased bone mineral density (Valimaki et al. 2004) but it is unclear as to whether it is associated with other bone geometric properties. A recent study in children found increased cortical thickness with increased 25(OH)D concentration (Sayers et al. 2012). There is a lack of such studies in adults, and none in South Asian populations. Any such changes in bone geometry could be an attempt of the bone to compensate for lower density. For that reason, it would be interesting to see if any differences in bone density and geometry, found at the radius and tibia, are associated with differences in vitamin D status, when controlling for relevant confounders (e.g. age, ethnicity, BMI).

1.4.4 Sleep health

1.4.4.1 Ethnicity, light, activity and rest-wake cycles

There has been some investigation into ethnic differences in rest-wake cycles and sleep quality, particularly concerning the characteristics and stages of sleep. For example, in polysomnographic studies a reduced amount of slow wave sleep (stage 3 and 4 NREM) and a longer sleep time has been found in African Americans compared with Caucasians (Stepnowsky et al. 2003) as well poorer sleep quality in Black men than Caucasian men (Ancoli-Israel et al. 1995). For self-reported sleep, it has also been found that African Americans may have higher self-rated sleepiness scores than Caucasian Americans (Sanford et al. 2006). Accordingly, another study found that African Americans have longer sleep duration and lower per cent of deep sleep than Caucasian Americans (Profant et al. 2002). The same result was also found in an actigraphic and polysomnographic study of older African American men by Song et al. (2012) who found longer sleep latency, higher sleep fragmentation, less slow wave sleep, less sleep efficiency and shorter total time asleep compared with same-age Caucasian men (Song et al. 2011).
In terms of other ethnic groups, polysomnographic studies have reported differences in slow wave sleep between Chinese and Caucasians (Hall et al. 2009). Two recent meta-analyses have assessed data collected worldwide on adolescent sleep, and found a clear variation in total sleep time, with Asian children (South and East Asia) having a shorter self-reported sleep duration (Olds et al. 2010) and later self-reported bedtimes (Gradisar et al. 2011) than European or American adolescents. Also, in adults, Jean-Louis et al. (2000) found a significant difference in actigraphic total sleep time and sleep efficiency by gender and ethnic group, with men from ethnic minority groups (Hispanic, Black or Asian) having the poorest sleep (Jean-Louis et al. 2000).

Genetic, physiological and social explanations have been proposed to explain these ethnic differences in sleep. First, genetic research has attempted to explain these differences by assessing differences in circadian clock genes by ethnic group. For example, one study found global differences in polymorphisms of the clock gene Arntl and Arntl2 (Ciarleglio et al. 2008). This clock gene produces a protein which hetero-dimerises with the Circadian Locomotor Output Cycles Kaput (CLOCK) gene protein, an important transcription factor involved in the timing of the circadian clock. Therefore, there could be a significant difference in the timing of the circadian clock among different global populations. Also, another study found that Asian populations have an increased frequency of the T allele of the CLOCK gene itself, as well as an increased frequency of the shorter form of the PER3 gene than Caucasians (Barbosa et al. 2010). This suggests potential ethnic differences in diurnal preference (‘morningness and eveningness’). Also, Smith et al. (2009) found that African Americans have a shorter endogenous clock length (tau), and differences in phase delay and advance than non-African Americans. Evolutionary mechanisms created by the effects of latitude could have generated these differences (i.e. a need for a shorter tau nearer to the equator) (Smith et al. 2009).

Second, other differences in physiology have been considered in relation to ethnicity, circadian rhythms and sleep. For example, it has been suggested that ethnic differences in eye colour may contribute to differences in melatonin production. One study found that Caucasians (blue, green or light brown eyes) had an increased melatonin suppression in response to a night time burst of 1000 lux (2 hours prior to peak melatonin concentration) than did Asians (Japanese, dark brown eyes) (Higuchi et al. 2007). Unfortunately, there was not a dark brown eyed Caucasian group to directly compare with the Asian group, so the real effect of ethnicity, independent of eye colour, could not be considered here. However, this study does suggest that ethnic differences in eye physiology might affect light transmission to the retina, and consequent circadian responses.

Last, socioeconomic status (SES) factors should also be considered. Social factors may affect sleep, and are known to vary by ethnic group. These factors may include poor housing in urban areas with
traffic noise disrupting sleep (Halonen et al. 2012), a lower level of education (Lallukka et al. 2012) and increased work related stress (Burgard and Ailshire 2009). Also, other biological factors that are influenced by social factors may also impact on sleep. These include obesity, exercise, and intake of caffeine and alcohol, all of which may affect sleep (Hartz et al. 2013).

It must be borne in mind that any investigations attempting to interpret the associations between social factors, ethnicity and reported sleep quality are complicated by likely ethnic differences in the reporting and awareness of sleep behaviour. These may be due to differing beliefs about ‘normal’ sleep and wakefulness and differences in the ability and willingness to report sleep difficulties. Also, the problem of ethnicity being associated with both health and socioeconomic status needs to be considered when interpreting this type of data.

As described in Section 1.3, light exposure is the primary zeitgeber of circadian entrainment. Accordingly, ethnic differences in light exposure are a likely candidate for explaining differences in sleep between ethnic groups. As discussed previously, Kripke et al. (2004) found that Caucasian women had a higher light exposure than Black or Hispanic women and that longer, but poorer quality sleep was associated with reduced light exposure (Kripke et al. 2004). Unfortunately, there has been no other research into the different amounts of light exposure by ethnicity and nearly all studies assessing ethnicity and sleep quality have been conducted in the USA, focussing on Black, Caucasian, Hispanic, and Japanese populations. Few studies have studied South Asian populations, and there is a lack of key data concerning light exposure and sleep patterns in this ethnic group. There are no known studies assessing the prevalence of sleep related problems in migrant South Asian groups in the UK or elsewhere in western society. Only a small amount of research has assessed sleep problems in South Asians, and these assessed only people residing in South Asia. Also, most sleep research in South Asians has been confined to studies of men, with sleep problems commonly being attributed to sleep apnoea, caused by obesity (Agrawal et al. 2011). One recent study that did assess both genders found that 10% of South Asian women (vs 8% of men) reported insomnia (Panda et al. 2012). However, there has been no research to assess whether insomnia rates, or sleep quality differs between Caucasian and South Asian women.

There are some potential reasons as to why South Asian women would be predicted to have poorer sleep than Caucasian women. First, health co-morbidities such as obesity, cardio-vascular disease and type II diabetes are more prevalent in South Asians than European populations (Brady et al. 2012; Cappuccio et al. 1997) and have been associated with sleep problems (Vgontzas et al. 2009). There are likely to be important social factors influencing sleep in South Asians. South Asians tend to be over-represented geographically in the lower socioeconomic (SES) areas of the UK than Caucasians, with 25% of Indian, 55% of Pakistani and 65% of Bangladeshi populations classified as in income poverty (Kenway 2007). Lower SES is known to be associated with poorer sleep
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(Lallukka et al. 2012; Arber et al. 2009), but this might not apply to all ethnic groups (Goodin et al. 2010) and ethnicity may have an SES independent association with sleep (Hall et al. 2009). Cultural practices may also disturb sleep, with sleep being disrupted during the Ramadan fasting period (Roky et al. 2001). It is unknown whether other religious customs have an effect on sleep, (e.g. Muslims awakening in the early morning hours for prayer).

Last, it is possible that low light exposure may play a role in the sleep of South Asian women. It is unknown whether western dwelling South Asian women have a lower light exposure, or go outdoors at different times of day (so receive differing amounts of light) than Caucasians. Also, there is a clear possibility that light exposure is lower in UK dwelling South Asian than Caucasian women. The recent Diet, Food Intake, Nutrition and Exposure to Sunlight in Southern England (D-FINES) study found that UK South Asian postmenopausal women have less UVB exposure than their Caucasian counterparts (Macdonald et al. 2011; Darling et al. 2013). Hence, it is likely that their total light exposure was also lower than Caucasians. Physical activity levels have been found to be correlated with sunlight exposure in older women (Grandner et al. 2006).

Of course, the results of the D-FINES study only predict lower overall sunlight exposure in South Asian women than Caucasian women. As discussed previously, for light exposure to entrain circadian rhythms, the time of day is very important. In the UK, light in the blue part of the spectrum is highest in the morning, and drops rapidly after noon (Thorne et al. 2009). Also, most people require a slight circadian advancement as their endogenous circadian length is slightly over 24 hours, and this is achieved by exposure to morning light. Interestingly, this is a slightly different time of day from that of peak UK vitamin D production, which increases from around 10am to midday, and falls off to a minimum at around 3pm. It is not clear whether individuals who are high in vitamin D would also be those receiving morning light. Therefore, it would be valuable to have light, vitamin D and sleep data on South Asian populations and to investigate any differences from Caucasians.

1.4.4.2 Vitamin D, light, musculoskeletal health and sleep

As described in Section 1.1, convincing aetiologies for the mechanism of vitamin D’s influence are being proposed for an increasing number of chronic diseases. Most recently, this includes an association between vitamin D and a variety of conditions linked to mental and behavioural disorders of the central nervous system. In particular, vitamin D has been associated with seasonal affective disorder (SAD) and low mood (Lansdowne and Provost 1998), depression (Anglin et al. 2013), autism and schizophrenia (Eyles et al. 2013). These associations are only speculative, and there is a clear possibility that the associations may be false, or that these problems might be a cause of vitamin D deficiency, rather than the result of it. It is particularly difficult to disentangle potential light related influences on SAD from those of vitamin D (Humble 2010).
Recent research has mapped the human brain to identify areas containing the vitamin D receptor (VDR) and vitamin D hydroxylase enzyme (Eyles et al. 2005). Despite interest in vitamin D function in the brain, and associations with mental illness, no research has specifically examined whether there is any link between vitamin D and circadian rhythms, or between vitamin D and sleep. The exception is one conference paper (Pande et al. 2009) which presented data on an association between poorer vitamin D status and shorter sleep duration in the USA. However, this study used NHANES survey data and so it was not possible to adequately control for light exposure. When assessing the association between vitamin D status and sleep it is crucial to control for the confounding factors of light exposure, physical activity and other factors such as musculoskeletal pain.

Historically, there have been some theories proposed for an influence of vitamin D on circadian rhythms and seasonal mood disorders. First, it has been proposed that vitamin D may have effects on circannual (‘year long’) rhythms. Maxwell (1994) discussed how UVB rays, along with other components of light, may have an effect on seasonal adaption in animals (Maxwell 1994). Maxwell (1994) also discussed how seasonal change in vitamin D itself may be linked to hormonal, behavioural and mood changes in animals. Although this is an interesting hypothesis, there is limited evidence to support it. Also, it must be borne in mind that even if plausible, it may not be relevant to humans, whose biological rhythms are less affected by seasonal change than animals.

A few years previously to Maxwell’s work, the researcher Walter Stumpf had proposed the theory that vitamin D is a ‘somatotrophic activator and modulator’ of biological systems (Stumpf 1988a) suggesting that vitamin D works in a similar way to melatonin and functions in conjunction with it (Stumpf 1988b). Melatonin is a sleep inducing hormone which is produced by the pineal gland during the hours of darkness. Therefore, a synergy between vitamin D and melatonin would imply that vitamin D also affects sleep in some way. Subsequent to this, Partonen (1998) suggested that 1,25(OH)₂D may be influential in the metabolism of melatonin and serotonin (Partonen 1998).

Aside from recent associations between vitamin D and mood disorders, little has been reported about the association between vitamin D status and biological rhythms since the late 1990s. Then in 2012, a paper reviewed the literature, and controversially suggested that vitamin D deficiency is a cause of sleep disorder (Gominak and Stumpf 2012). The paper made very strong claims that are not yet fully substantiated by the evidence base. Much more research is required in this area, not least good quality vitamin D supplementation trials to investigate the effects of vitamin D on circadian or rhythms, and sleep quality. However, this recent review did reawaken interest in the vitamin D and biological rhythms hypothesis. The focus now turns to the recent, and important, discoveries that
have been made regarding the role of vitamin D, and the vitamin D receptor (VDR) in the brain, and makes the argument for further investigation of the vitamin D-sleep hypothesis.

First, the VDR is a nuclear steroid receptor and it is ubiquitous in the human brain (Eyles et al. 2005). For activity, it must form a heterodimer with the retinoic acid receptor (RXR) which has vitamin A (retinoic acid) as its ligand. The VDR enables transcription of Vitamin D response elements, which contain genes for a variety of cell functions, mainly concerned with cell proliferation and differentiation. It is not known whether the VDR is involved in biological rhythms, but it is of interest that other nuclear receptors (e.g. Retinoic acid related orphan receptor; ROR) have been shown to have a clear role in biological rhythms (Jetten et al. 2001). The ubiquitous presence of the vitamin D receptor (VDR) in the brain means that it is likely that vitamin D is active in some of the brain areas controlling biological rhythms. McGrath et al. (2004) discuss how the VDR is expressed in the thalamus, cortex and hippocampus (McGrath et al. 2004), brain areas which are also known to control of sleep, memory and wakefulness (reviewed by Brown et al. 2012).

Second, vitamin D has recently been termed a ‘neurosteroid’ (McGrath et al. 2001). Neurosteroids have been found to affect memory and sleep in rats (George et al. 2006) and it is tentatively possible that vitamin D may also have such functions. Third, there is a particularly high concentration of the VDR in the hypothalamus, especially in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) (Eyles et al. 2005). The PVN is involved in the transmission of impulses from the SCN to the pineal gland. VDR concentrated areas of the brain may be directly or indirectly involved in, or interact with circadian pathways. Fourth, it has been suggested that 1,25(OH)2D may increase serotonin concentration by preventing nuclear retinoid Z receptors binding melatonin as a ligand (Partonen 1998). Hence, adequate vitamin D status may reduce the risk of SAD (Partonen 1998). Last, a recent study in rats has shown that a molecule called Calbindin-D28K, regulated by 1,25(OH)2D, may be involved in the entrainment of the circadian rhythm to light (Stadler et al. 2010). Thus, there may be a specific mechanism for a potential influence of vitamin D on circadian rhythms. However, there are some problems in this interpretation, with some evidence suggesting that it is only renal calbindin-D28K, not brain calbindin-D28k, that is responsive to 1,25 (OH)2D (Varghese et al. 1988). More data is required to resolve this issue, but the fact that vitamin D only affects the expression of renal calbindin-D28k, and not the expression of the brain’s version of this protein, suggests that vitamin D may not affect the entrainment pathway after all, at least in rats.

There are some clear contradictory observations that must be borne in mind. VDR null rodents have been found to display a variety of abnormal behaviours, including abnormal grooming (Kalueff et al. 2004) and locomotor behaviour (Burne et al. 2005). However, they have not been reported to show abnormalities in circadian rhythms or rest-wake behaviour, although it could also be argued that no studies have explicitly set out to assess whether VDR null mice have abnormalities in sleep wake
Introduction

It is important that any effect of vitamin D status on biological rhythms is elucidated, due to the importance of biological rhythms for daily functioning and health, and the widespread prevalence of vitamin D deficiency both in the UK and globally. Preliminary epidemiological investigations are now required to assess whether such a relationship exists and, in particular, to fully investigate the interrelationships between vitamin D status, light exposure, musculoskeletal problems and sleep. Sleep is known to be affected by musculoskeletal fatigue and pain (Rohrbeck et al. 2007) which in turn could lead to reduced outdoor activity (and less light and vitamin D), which may detriment health further, setting up a vicious cycle. It would also be interesting to see if there are ethnic differences in these inter relationships.

1.5 Social influences on sun exposure

A major omission in the vitamin D literature concerns the sociological aspects of vitamin D deficiency. Due to sun exposure being the main determinant of vitamin D status, the social influences on sun exposure and the cultural beliefs, perceptions and attitudes that influence this are likely to be important. These factors include perceptions of skin colour and beauty, perceptions of health risk and other potentially relevant sociological influences influencing sun exposure behaviour in different ethnic groups.

1.5.1 Sun avoidance in Muslim and Hindu populations

There is a lack of research assessing sun exposure in Muslim and Hindu populations. The little research that has been conducted has been undertaken in a variety of different countries, religious and ethnic groups and age and gender sub-populations, making generalisations difficult.

Many Muslim and Hindu women have been found to practice sun avoidance, at least to some degree. For example, in South Asian women living in New Zealand, widespread sun avoidance has been found, with common concerns stated about sun intensity and skin cancer risk (von Hurst et al. 2009). In young Arabian women, Thomas et al. (2010) found that covering of the body and head and skin tone preferences were an important factor influencing sun exposure, as well as strong beliefs in the dangers associated with sun exposure (Thomas et al. 2010). The above two studies both used a quantitative approach via rating scales on questionnaires.

Sun avoidance is also found in some white women and may affect their vitamin D status. For example, N-HANES 2003-2006 data showed that US Caucasian women who wore long sleeves and sat in the shade had reduced 25(OH)D concentration compared to those who did not (Linos et al.
Conversely, even in Muslim and Hindu populations, sun avoidance is not universal. For example, a study in Morocco found that over half of female study participants spent over 2 hours in the sun per day, around midday (Abda et al. 2012). Also, sun protection devices or strategies (e.g. hat, suncream, and sitting in the shade) were infrequently used (Abda et al. 2012). Similarly, a recent study found that a sun exposure of around 1-2 hours per day was common in Pakistani women (Mahmood et al. 2009) whilst another study reported that the average time spent in the sun by university workers and students in Pakistan was 70 mins (indoor staff), 84 mins (students) and 329 mins (outdoor staff) (Humayun et al. 2012).

Overall, sun avoidance appears to be common in Muslim and Hindu women. However, sun avoidance in most cases is likely not complete, and sun exposure behaviour, and use of sun protection varies widely within Muslim and Hindu populations. It is also likely that migrants living in western societies behave differently to their peers dwelling in the country of origin and it is important to assess the sun exposure of migrants in addition to that in their home country. More research is required to specifically assess sun exposure behaviour in South Asian women living in the UK, due to their high risk of vitamin D deficiency.

1.5.2 Ethnicity and skin tone preferences

Given the evidence discussed that some degree of sun avoidance is practiced by women in Muslim and Hindu cultures, it is important to elucidate the possible social factors explaining this phenomenon. One possible important factor is the influence of perceptions of beauty and skin colour in determining sun exposure. It is important to understand these beliefs, as a desire for tanning (or not) may influence vitamin D status through alterations in sun exposure behaviour.

In many non-Caucasian cultures pale skin is seen as representative of beauty. For example, Japanese cultures perceive whiteness as symbolic of group identity as ‘being Japanese’ (Ashikari 2005). In African populations lighter skin colour is associated with higher status and increased beauty (Charles 2009). Such perceptions in Black populations have been suggested to stem from colonial history where ideologies of lighter skin being associated with higher status may still persist (Hunter 1998). Similarly, in South Asian populations darker skin tone is often perceived as undesirable (Grewal 2009), and these ideologies may still continue, even when the populations have migrated away from the country of origin (Grewal 2009) and when these distinctions are less socially relevant.

Preferences for a lighter skin tone have been associated with the use of skin lighteners (Hamed et al. 2010). There is concern about the use of skin lighteners because they contain bleach, which can cause skin damage. Skin lighteners are widely available in South Asia, and widely used, both
commercially purchased and home-made according to traditional recipes (Hussain 2005). Skin lighteners are also being increasingly used in Middle Eastern (AlGhamdi 2010) and African populations (Dadzie and Petit 2009).

These preferences for a pale appearance are in stark contrast to those of White Caucasians, who are known to have a preference for a tanned appearance. For example, a tan has been seen as portraying healthiness (Clarke and Korotchenko 2009) and attractiveness (Sahn et al. 2012). This desire in Caucasians for a tan is likely to have some roots in the portrayal by Western media of beauty. An analysis of Western fashion magazines from the early twentieth century suggested a growing interest in tanning emerged in the late 1920s, with a corresponding decrease in the interest in, and promotion of pale skin (Martin et al. 2009). A recent study in young Caucasian Australian women, showed that increased exposure to images of ‘tanned’ models was associated with increased beliefs that a suntan is ‘desirable’ (Dixon et al. 2011). In older Caucasian women, exposure to these same images was associated with increased tanning behaviours, but surprisingly not positive attitudes to tanning (Dixon et al. 2011). It is possible that the lack of positive attitudes reported by these older women may have been due to reluctance in reporting, as their subsequent behaviour suggests they did feel it was a desirable thing for them to do (Dixon et al. 2011).

Recently, there has been an increased availability of ‘sunless’ tanning products (i.e. ‘fake’ tans). A recent study suggests the use of these products may have led to a decrease in UVB radiation exposure, from both natural sun and artificial sunbeds (Sahn et al. 2012). This would have benefits for reducing skin cancer risk, but may not be beneficial for vitamin D status, particularly as those using fake tans tend to be paler in complexion (Sahn et al. 2012). This may be a problem as pale skinned Caucasians are known to be at increased risk of vitamin D deficiency (Glass et al. 2009).

Despite the above preference for paler skin in many ethnic minority groups, other factors may also be important in determining sun exposure behaviour. In one study, religious and cultural observations, rather than avoidance of skin darkening, remained the main reasons cited for body coverage in adolescent girls with olive or medium skin tones (Fairbanks et al. 2012). Of note, most dark skinned girls did not cite wanting to prevent darkening as a reason for body coverage, even when allowed to give multiple reasons for their choices (Fairbanks et al. 2012). It must be borne in mind that this result may be specific to the age of the study participants and to the ethnic groups present in the London borough where the study was conducted. Indeed, these conclusions may not apply to all ethnic groups, including many South Asians. Nonetheless, this suggests that skin tone is not necessarily the main reason for a modest dress style and perhaps not for sun avoidance either. Other cultural factors may be as, if not more important than skin tone preferences in explaining sun exposure behaviour in ethnic minority groups, such as South Asians. These other factors will now be discussed.
1.5.3 Work, Family and Leisure in South Asian women

There is some limited evidence that it may not always be deliberate sun avoidance that is responsible for poor vitamin D status in populations. In a Californian study of White Caucasian men, women and children, low levels of avoidance of the sun was found, with only 22% regularly using sunscreen (Hoegh et al. 1999). This study highlighted indirect avoidance such as an indoor working and leisure lifestyle (Hoegh et al. 1999), which even in hot climates has been found to reduce vitamin D status (Azizi et al. 2009). This would explain the worldwide prevalence of vitamin D deficiency, even in countries where the UVB is of sufficient wavelength all year round to promote vitamin D production and high overall UVB irradiance.

Lifestyle commitments may use up time that could be used for sun exposure. As members of communities, many South Asian women feel a sense of responsibility to help improve the provision of community services (‘khidmat’) (Bolognani 2013). This takes the form of unpaid community support, which may take up much of their free time. It must be remembered that older South Asian women in the UK are a population of first generation migrants, who originally entered the UK in the 1950s and 60s to re-join their migrant husbands. Although originally supposed to be a ‘temporary’ arrangement, to boost economic wealth, for many Asians this has become permanent. Over time, as the South Asian communities resided in the UK longer, they stayed permanently. There are likely to have been some adaptations in behaviour and customs to adjust to living in British culture. Despite this, Hussain (2005) discusses how being attached to the homeland still impacts on behaviour, including keeping original cultures and traditions alive, sending money to relatives, and using imported cultural items from the land of origin (Hussain 2005).

A need to keep cultural traditions means South Asian women are likely to be following to some extent a similar lifestyle to that of South Asia. In terms of sun exposure this might include avoidance of outdoor activity in the middle of the day, even though the midday sun is not as hot in the UK as in South Asia. The strong ties to the homeland also means for many South Asian women regular trips home to see family, friends and the community of their origin. These trips are seen as very important, and usually occur once a year, for a few weeks duration. Likely restrictions on visits to South Asia include responsibilities (e.g. work) in the UK and lack of finances for repeated trips. These trips mean that there may be no time or money for, or no need for, other holidays. This is unlike Caucasians, who will often have one (or more) holidays abroad each year, as well as short duration trips to see friends and family. These ethnic differences are likely to have consequences for sun exposure behaviour and vitamin D status.
Immigrants face the task of maintaining their own ethnic identity and practices, whilst aiming to ‘fit in’ with the new society they have moved into (Choudhry 2001). For some South Asian groups, this may include limited outside activity, with most daily activity being reasonably sedentary but with clear purpose, including visiting friends or relatives in the community. This is in contrast to Caucasians who may do physical activity for other reasons (e.g. fitness), or who are more likely to sit outdoors for relaxation without a clear, practical purpose. Many South Asian community activities involve indoor work, (e.g. indoor festivals, help at the mosque or temple) rather than outdoor activity. South Asian women feel a duty to help others, and may feel putting themselves first to be irresponsible (Hussain 2005). Hence, they may lack time for their own health (e.g. relaxation in the sun). The lack of leisure time and feelings of duty towards family, friends and the South Asian community are likely to reduce their ability to increase sun exposure time. In addition to unpaid responsibilities, many South Asian women are in part or full-time paid work. This again leads to little time being available for leisure purposes.

Asian migrants may also take on some habits and customs of the local UK population. Changes have been seen on the South Asian continent, whereby levels of physical activity are reducing, due to increased urbanisation and adoption of a more western lifestyle. Of concern, average physical activity levels are now lower in urban India than in the USA and UK (Sullivan et al. 2011). Interestingly, the Sullivan et al. (2011) study examined recent rural to urban migrants within India. They found that rural to urban migrants resembled the local urban population in physical activity behaviours more than rural populations, suggesting the current societal context might be more important than previous life experiences in affecting physical activity (Sullivan et al. 2011). Consequently, it is likely that migrant South Asian women are likely to have adopted current UK physical activity patterns, at least to some extent. Therefore, it can be assumed they are most likely reasonably sedentary.

The problems of social space and dress style in urban living environments may also be influential on South Asian women’s ability to expose skin to the sun. An Australian study found that urban dwelling South Asian women lacked what they perceived as ‘safe’ locations to get sun exposure (e.g. due to being over looked in high rise flats) (Brand et al. 2008). This is likely to be more of a problem for South Asian than White women due to the strict requirement for a covered dress style at all times in public. If living quarters (e.g. gardens) are overlooked by other dwellings then South Asian women are unlikely to feel comfortable exposing skin to the sun. The wearing of religious dress is an important symbol that the woman adheres to the community’s behavioural norms and values (Hussain 2005). Thus, it would not be feasible to suggest changes in dress style to allow improvements in vitamin D status. This is unlike Caucasians, who have more flexibility over their dress style. Also, as described in Section 1.4.1.4, most South Asian diets do not contain much
vitamin D, (the exception being Bangladeshi diets which contain a large component of oily fish). Moreover, as discussed previously, vitamin D supplements are not usually taken.

To summarise, for South Asian women, it is likely that religious and cultural factors including preference for a pale skin tone, a covered dress style, a diet low in vitamin D, a large number of caring responsibilities (paid and unpaid), an indoor lifestyle, annual travel to meet family in South Asia rather than leisure holidays per se, as well as low use of vitamin D supplements are likely to lead to poor vitamin D status. This reduced sun exposure is also likely to lead to reduced light exposure, which may have negative effects on health. Morning light exposure has been positively associated with quality of life in older women (Grandner et al. 2006). Similarly, total light exposure has been found to be associated with social functioning, quality of life, and emotional wellbeing, even when physical activity is controlled for (Grandner et al. 2006). Therefore, the traditional South Asian way of life may impact detrimentally on vitamin D status and light exposure, and potentially on health. These problems are likely to be compounded by lack of support from the relevant health agencies to get help or advice regarding vitamin D. In the study by Brand et al. (2008) western dwelling South Asian women reported problems finding written information in their own languages, and they also had problems accessing vitamin D supplements. However, no previous research in South Asian populations has studied sunlight exposure in the context of vitamin D production and exposure to light for sleep health. This is a key area which needs to be studied, due to the potential detrimental health consequences of low vitamin D status and poor light exposure.

1.6 Overview of the Thesis

1.6.1 Outlining and reformulating research problem, purpose and scope

The task to be addressed in this Thesis is that of enhancing the understanding of biological and social influences on low vitamin D levels in UK dwelling South Asian women, and the likely health effects of this. The purpose is to provide key information to inform public health providers in order to make interventions to increase vitamin D status more effective in South Asian women, in order to reduce any adverse health effects. This will be achieved by providing key data to fill in some of the important gaps in the literature. These gaps relate to both the biological and social influences on vitamin D status, as well as the adverse health outcomes arising as a result of vitamin D (and sunlight) deficiency.

As discussed previously, the biological causes of low vitamin D are reasonably well known in South Asians, but there are still some key omissions in the literature base. One clear gap in the research
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concerns whether seasonal change in vitamin D, rather than just average vitamin D concentration affects bone health. This is important, as it will enable understanding as to whether it is better to supplement with vitamin D all year around, or just in winter to blunt the rhythm of seasonal change. Also, there is a lack of knowledge about the change in vitamin D status over a period of years, and the predictors of this change. This knowledge will enable the tailoring of interventions to target the most influential biological factors, giving a more effective improvement in status.

In contrast, the social factors influencing sun exposure in South Asian women, and vitamin D status, are not clear. Possible factors such as skin tone preference and religious and ethnic influences on family, work and community life have been identified as potential explanations for the lower vitamin D status in South Asian women, via lower sun exposure. Further elucidation of the social factors influencing sun exposure could help to design interventions to effectively, and safely increase sun exposure in South Asian women.

Another major omission in the literature is whether vitamin D deficiency in South Asians is accompanied by a low light exposure. As light exposure is important for sleep quality, it would be predicted that if light exposure was lower in South Asians, then their sleep health could be adversely affected. Just as has been the case for light exposure in older, institutionalised people, and with shift workers, knowledge about light exposure in South Asian women may help identify further areas for research and encourage development of interventions to improve light exposure if it is found to be inadequate. In tandem with this, it is important to elucidate whether vitamin D deficiency is associated with poorer sleep, as if so, improvement in vitamin D status could be trialled as a way of improving sleep health in individuals of all ethnic groups.

Last, it is not clear whether South Asians have poorer musculoskeletal health than Caucasians and whether musculoskeletal health is associated with vitamin D status. It is still unresolved as to whether South Asian women have poorer bone health than Caucasian women, and their bone geometry has been little studied. This information would be very valuable in assessing potential therapies for osteoporosis and providing a correct assessment of fracture risk for South Asian women.

This Thesis aims to investigate the inter-relationships between ethnicity, vitamin D status, light exposure, musculoskeletal and sleep health. It uses a multi-disciplinary approach, combining contributions from the disciplines of Nutrition, Chronobiology and Sociology, and using both quantitative and qualitative data analysis methods.

The scope of the research was limited to Southern UK dwelling South Asian and Caucasian women of any religious affiliation and country of migratory origin. For the main analysis, the scope was
restricted to the postmenopausal women. For the light and sleep sub-study, due to the likely
difficulty in recruiting sufficient postmenopausal women, both postmenopausal and premenopausal
women were included. For the social data the scope was restricted to the oldest South Asian and
Caucasian postmenopausal women (60-70 years). This was because qualitative data analysis required
a tighter restriction on the characteristics of the women in order to provide meaningful data. The
scope of the information that was obtained is illustrated in Figure 1.7, presented as a schematic to
emphasise the relationships being tested as well as the main subject areas.

In summary, this research aims to help fill the gaps in the literature regarding social factors
influencing vitamin D status in South Asians, whether South Asians have lower light exposure than
Caucasians, and whether vitamin D deficiency is associated with musculoskeletal health and poorer
sleep health in both ethnic groups. This overarching aim is split to 9 smaller aims. These aims and
their corresponding null and alternative hypotheses (if applicable) are outlined in Section 1.6.2.

Figure 1.7: A proposed schematic for investigating the problem of poor vitamin D status and its
health consequences in South Asian women

1.6.2 Aims and Hypotheses

Aim 1- To assess vitamin D status in South Asian and Caucasian women, including its seasonal
stability.

Alternative Hypothesis 1: South Asian women will have lower vitamin D status, but more stable
25(OH)D throughout the year than Caucasian women
Null Hypothesis 1: There will be no difference in vitamin D status and stability of 25(OH)D
throughout the year by ethnicity.
Aim 2 - To assess whether there are differences in indices of bone health by degree of seasonal fluctuation of 25(OH)D status (independent of the confounding effects of actual 25(OH)D status, diet and lifestyle factors).

Alternative Hypothesis 2: Women who show higher seasonal fluctuation will have will have poorer bone health than those who show less seasonal fluctuation. 
Null Hypothesis 2: There will be no difference in bone health by degree of seasonal fluctuation of 25(OH)D.

Aim 3 - To assess change in vitamin D status over a period of four years (2006-2010) and to determine which the relative contributions of factors (change in UVB, change in vitamin D intake, change in calcium intake, change in BMI, age and ethnicity) in predicting change in vitamin D status.

Alternative hypothesis 3: Change in UVB, change in vitamin D intake, change in calcium intake, change in BMI, as well as age and ethnicity, will be significant predictors of change in 25(OH)D (from 2006-2010).
Null hypothesis 3: Change in UVB, change in vitamin D intake, change in calcium intake, change in BMI, as well as age and ethnicity, will not be significant predictors of change in 25(OH)D (from 2006-2010).

(Aims 1 -3 are addressed in chapter 3)

Aim 4 - To assess the musculoskeletal health of the two ethnic groups

Alternative Hypothesis 4: South Asian women will have lower volumetric bone density, poorer lower extremity muscle function and higher pain scores than Caucasian women.
Null Hypothesis 4: There will be no difference in volumetric bone density, lower extremity muscle function and pain scores by ethnic group.

Aim 5 - To assess whether vitamin D status is associated with measures of bone geometry and muscle strength

Alternative Hypothesis 5: There will be an association between vitamin D status and bone geometry and muscle strength.
Null Hypothesis 5: There will be no association between vitamin D status and bone geometry and muscle strength.
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(Aims 4 and 5 are addressed in chapter 4)

Aim 6- To assess whether South Asian women have different light exposure and sleep quality than Caucasian women

Alternative Hypothesis 6: South Asian women will have lower light exposure and poorer sleep quality than Caucasian women
Null Hypothesis 6: There will be no significant difference in light exposure and sleep quality by ethnicity.

Aim 7- To assess whether there is an association between vitamin D and light exposure in the two ethnic groups

Alternative Hypothesis 7: There will be a significant association between vitamin D status and light exposure in the two ethnic groups
Null Hypothesis 7: There will be no significant association between vitamin D status and light exposure in the two ethnic groups

Aim 8- To assess whether there is a link, (independent of light exposure, lifestyle, personal factors and musculoskeletal pain), between vitamin D and sleep quality

Alternative Hypothesis 8: There will be a significant association between vitamin D status and objective and subjective sleep quality
Null Hypothesis 8: There will be no significant association between vitamin D status and objective and subjective sleep quality

(Aims 6-8 are addressed by chapter 5)

Aim 9- To assess qualitatively the attitudes, beliefs, perceptions and behaviours surrounding sun exposure in UK South Asian and Caucasian women.

(Aim 9 is addressed by chapter 6)
1.6.3 The value and originality of the research

The value and originality of this work stems from both the multidisciplinary approach used and the unique subject areas being investigated. First, the subject matter is original as most previous work on social influences on sun exposure behaviour has been done in Caucasian rather than South Asian women. Also, most work has been done in the USA or Australia, not the UK, and very little of this research has also assessed vitamin D status. Nearly all research has been in younger women, children or adolescents, rather than postmenopausal women. In all ethnic groups there is a lack of research directly looking at attitudes and beliefs about sun exposure, especially in relation to vitamin D, with very little, if any, qualitative research done. Understanding social influences is essential for the production of effective public health campaigns. This is because interventions may need to be tailored to the needs of different ethnic minority groups to be effective in their goals.

Second, there are no known data, previous to the data collected here, on bone geometry in older South Asian women. So, it is unknown whether there are ethnic differences between South Asian and Caucasian older women in bone health. It is also unclear whether vitamin D has effects on bone geometry and muscle strength, beyond that of bone density. It is important to understand bone geometry and muscle strength to assess likely fall and fracture risk in different ethnic groups. Understanding the relationship between vitamin D and musculoskeletal health is important to assess whether improving vitamin D status is likely a useful strategy for improving musculoskeletal health.

Third, there are also no known data on light exposure in either South Asian men or women, and no data on sleep patterns in South Asian women specifically. It is valuable to understand light exposure and sleep health in all ethnic groups to produce interventions to improve sleep health, and assess which ethnic groups are at highest risk of sleep problems, and targets for interventions. Also, it is unknown whether sleep is associated with vitamin D status, as despite some speculative theory, and proposed biological mechanisms, there is no published data to date in any ethnic group assessing this relationship. It is important to assess whether there is a relationship between vitamin D and sleep health, as if so, vitamin D supplementation may be helpful in reducing the burden of poor sleep to individuals and society.

Last, it is unknown whether seasonal change in vitamin D status impacts on bone health. Understanding whether seasonal change in vitamin D status is associated with musculoskeletal health is also important in the decision as to whether vitamin D should be supplemented all year around, or just in winter to ‘blunt’ the rhythm. Also, there is very little knowledge as to the predictors of long term change in vitamin D status over a period of years. Understanding the factors influencing change in vitamin D over a period of years is also useful in tailoring interventions to target the most influential factors effectively to improve vitamin D status in populations.
CHAPTER 2- Methodology
2.1 The D-FINES study (Summer 2006 - Spring 2007)

2.1.1 Study design and recruitment

The D-FINES (Vitamin D, Food Intake, Nutrition and Exposure to Sunlight in Southern England) study was run at the University of Surrey from June 2006 to May 2007. In this study, subjects visited the University of Surrey four times over the course of one year, once in summer (June to August 2006), once in autumn (September to November 2006), once in winter (December 2006 to February 2007) and once in spring (March to May 2007). A summary of the purpose, design and methodology of the original D-FINES study is in Appendix A.

Study participants had been recruited from twelve local GP surgeries spread over the South East of England, including Guildford, Woking, Basingstoke, Aldershot, Croydon, Kingston, Thornton Heath and outer London. Potential subjects from the electronic databases at these GP surgeries had been mailed a letter inviting them to take part in the study. These women, randomly chosen for an invite, were from those that had a close match to the inclusion criteria, as well as being deemed not likely to match the exclusion criteria (Table 2.1). The women were all of Caucasian or South Asian ethnic origin. By this method, n=135 Caucasian premenopausal and n=144 Caucasian postmenopausal women were recruited to the study. However, this method was not successful for the recruitment of South Asian women as none of the women contacted offered to take part. Consequently, the Principal Investigator of the study contacted local South Asian women’s networks. This enabled the recruitment of n= 46 premenopausal Asian and n= 40 postmenopausal Asian women. This gave a total of n=375 women. See Figure 2.1 for details of participant flow through the study.

In accordance with the ethical standards laid down in the 1964 Declaration of Helsinki, a favourable ethical opinion was obtained from relevant Research Ethics Committees (National Health Service NHS REC 06/Q1909/1 and the University of Surrey EC/2006/19/SBMS). Written, informed consent was obtained from all participants.

2.1.2 Power calculations

Power calculations for the D-FINES study had been based on two main aims. The first was to compare the postmenopausal Caucasian women’s 25(OH)D status to those of postmenopausal women attending a similar study being run concurrently at the University of Aberdeen (Aberdeen Nutrition Sunlight and Vitamin D study).
Table 2.1: Inclusion and exclusion criteria for the D-FINES study (2006-2007)

<table>
<thead>
<tr>
<th><strong>Inclusion</strong></th>
<th><strong>Exclusion</strong></th>
</tr>
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<tbody>
<tr>
<td>South Asian or Caucasian ethnicity (self-reported)</td>
<td>Taking prescribed steroids or other medication likely to affect vitamin D metabolism</td>
</tr>
<tr>
<td>Premenopausal or Postmenopausal Status</td>
<td>History of any disorder of calcium homeostasis or diagnosed osteoporosis</td>
</tr>
<tr>
<td>Age 18-65, female</td>
<td>Currently undergoing menopausal transition</td>
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<tr>
<td></td>
<td>Abnormal liver or kidney function</td>
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<tr>
<td></td>
<td>Currently on hormone replacement therapy (HRT) or other medications affecting bone metabolism (e.g. bisphosphonates).</td>
</tr>
<tr>
<td></td>
<td>Likely to have frequent travel to sunny climates during year of study</td>
</tr>
<tr>
<td></td>
<td>Currently pregnant or breastfeeding (or planning to become pregnant in next 12 months)</td>
</tr>
<tr>
<td></td>
<td>Taking vitamin D supplements; and not able/willing to refrain from use 3 months prior to and during study period</td>
</tr>
</tbody>
</table>

Power calculations for the Surrey Caucasian women were based on the numbers required to show a 0.4 standard deviation (SD) difference between subjects living in the South of England, and those living in Aberdeen (Macdonald et al. 2011). This gave an estimate of n=78 Caucasian participants required to give 80% power. Twenty-five per cent extra participants were added to allow for potential dropout, giving a requirement of n=135 Caucasian premenopausal and n=135 postmenopausal women (Macdonald et al. 2011). The full published paper of Macdonald et al. (2010) is shown in the Publications Appendix.

The second aim of the D-FINES study was to compare the 25(OH)D status between Surrey Caucasian and South Asian women. A previous study reported a mean 25(OH)D status in South Asian women of 6.9ng/ml with an SD of 7.1ng/mL (Pal et al. 2003). Power calculations using these values suggested that, for 80% power, n=19 premenopausal and n=19 postmenopausal South Asians were required to detect 0.8SD difference between South Asians and Caucasians (Macdonald et al. 2011). Forty-five per cent extra Asian participants were recruited, to allow for subject dropout. This gave a recruitment target of n=39 premenopausal and n=39 postmenopausal Asian women (Macdonald et al. 2011).
Methodology

source: (Macdonald et al. 2011)

Figure 2.1: Flow chart for recruitment in original D-FINES study 2006-2007

2.1.3 Study procedure

At the baseline study visit (summer), participants gave a fasting blood sample for measurement of serum 25(OH)D, serum parathyroid hormone (sPTH) and serum C-telopeptide of collagen (sCTX). This blood sample was also analysed for serum albumin and calcium to detect any primary hypothyroidism in the participants, which would exclude the participant from further study participation. Liver and renal function tests were also performed to check for undiagnosed liver or renal abnormalities, which would also exclude the participant from continuing in the study. After blood samples were obtained, participants were given breakfast. After this, participants performed grip strength tests using a grip strength dynamometer. They also completed physical activity and food frequency questionnaires. Then, after the visit, they were asked to complete a 4 day food diary (including one weekend day). They were also asked to wear polysulphone dosimeter badges, to assess sun exposure, for 7 days (one badge for 5 weekdays, and one badge for 2 weekend days). These badges were pinned to the lapel (or closest position) of their outer most layer of clothing. The participants returned both the food diary and the dosimeter badges to the University by post. At the autumn and spring visits, all participants travelled to the local hospital for a DXA scan to assess body composition, aBMD and areal Bone Mineral Content (aBMC). A subset of participants (n=80
Caucasian; n=41 Asian) also underwent a Qualitative Ultrasound (QUS) scan of the calcaneus to measure Broadband Ultrasound Attenuation (BUA) and Velocity of Sound (VOS).

2.1.4 Biochemical analysis

Serum 25(OH)D and sPTH were measured by the Vitamin D Research Group at the University of Manchester as described in detail previously (Darling et al. 2013) The full published paper of Darling et al. (2013) is shown in the Publications Appendix. The above laboratory participates successfully in the Vitamin D External Quality Assessment Scheme (DEQAS) and is accredited to quality measurement standards ISO 9001:2008 and ISO 13485:2003). Briefly, serum 25(OH)D was measured using the manual Immunodiagnostic Systems (IDS) enzyme immunoassay (Boldon, Tyne and Wear, UK) (Darling et al. 2013). Manufacturer’s reference ranges were 19-58 ng/ml (48-144 nmol/L) but vary with season; sensitivity 2 ng/ml (5 nmol/L); intra- and inter-assay coefficients of variation (CV) 6% and 7%, respectively (manufacturer’s values). Serum intact parathyroid hormone (PTH) was measured using the IDS OCTEIA immunoenzymometric assay (Boldon, Tyne and Wear, UK). The normal adult reference range is 0.8-3.9 pmol/L; sensitivity 0.06 pmol/L; intra- and inter-assay CV 4% and 6%, respectively (manufacturer’s values) (Darling et al. 2013). Serum CTX was measured using an electrochemiluminescent immunoassay (Roche cobas e411 automated analyser) at the University of Sheffield (Metabolic Bone Centre, University of Sheffield Medical School, UK). Intra-assay CV was: 5.7% (n = 12, mean 0.19 ng/mL). Inter assay CV was: Level 1 QC: 2.1% (n = 9, mean 0.30 ng/mL); Level 2 QC: 3.6% (n = 9, mean 0.70 ng/mL); Level 3 QC: 6.6% (n = 9, mean 2.86 ng/mL). 25(OH)D and PTH were measured for all participant samples, but due to cost restrictions, the bone marker, serum C-telopeptide, was only measured in a subgroup of n = 65 women (South Asian, n = 30 and Caucasian, n = 35). These women were randomly selected from all the women who had successfully attended all four visits.

2.1.5 Analysis of food diaries and dosimeter badges

The food diaries were analysed using the WinDiets programme (Research Version, Robert Gordon University). The dosimeter badges were read using a spectrophotometer at the University of Surrey (CECIL Aquarius CE7200). The following formula was used in an excel spreadsheet to convert absorbance at 330nm, as read by the spectrophotometer, into Standard Erythemal Dose (SED)\(^2\):

\[
\text{SED} = 10.7 \Delta A_{330} + 14.3 \Delta A_{330}^2 - 26.4 \Delta A_{330}^3 + 89.1 \Delta A_{330}^4
\]

This gave a reading in SED for each individual’s weekday and weekend badges in each season.

\(^2\) 1 SED = erythemal effective radiant exposure of 100 Jm\(^{-2}\) (100 Joules per metre squared).
2.2 Collection of new data: Summer 2010

2.2.1 Contacting previous participants

The first stage of recruitment in 2010 was to re-contact the participants who had attended the D-FINES study in 2006-2007. This was to enable longitudinal comparisons with the previously collected summer 2006 data. In May 2010, all subjects in the original D-FINES cohort were sent a letter inviting them to attend one of a series of presentations and to participate in the new study (see Appendices B and C for recruitment information). Figure 2.2 illustrates the flow of participants through the 2010 study.

Recruitment presentations were held at the University of Surrey, and at the Asian Women’s Network Centres. Participants were also encouraged to invite female friends and relatives to attend the presentations some of whom subsequently volunteered to take part in the study. This led to n=81 postmenopausal (n=20 Asian and n=61 Caucasian) and n=32 premenopausal participants (n=13 Asian and n=19 Caucasian) taking part in the main study.

2.2.2 Main study protocol

The study protocol and layman’s summary can be seen in Appendices D-E. The main study consisted of one summer visit, designed to mirror the original D-FINES study, in order to enable valid longitudinal analysis. Study procedures were as similar as possible to that of the 2006 study but with some differences, which are described here. A pQCT scanner was available offering a technological advancement on the previous methods and so was substituted for DXA and QUS. Also, stand to walk tests and the muscle and bone and muscle pain questionnaire were undertaken (see Appendix F for details of this questionnaire). Justification for the use of these muscle function tests and the questionnaire is given in Section 2.2.5 – 2.2.6. As in 2006-2007, subjects kept the same 4 day food diary after their visit, and wore two dosimeter badges over one week. The same health, lifestyle, and physical activity questionnaires were used as in the original study (see Appendices G-K for details of the food diary and these questionnaires) with the addition of questions about caffeine and alcohol intake, as well as usage of sleep medications. These extra questions were required to analyse data from the participants taking part in the light and sleep sub-study analysis. Extra questions were asked about holidays in the previous year (see Appendix J). Participant instructions for the use of the diaries and dosimeters are in Appendix L.
Biochemical analysis procedures, as well as analyses of food diaries and dosimeter badges were identical to that of the original 2006-2007 study (see Section 2.1.4 and 2.1.5). The exception to this was the measurement of 25(OH)D by High Performance Liquid Chromatography (HPLC) as well as by immunoassay. Justification for this decision is given in Section 2.3.1.

Ethnic origin was self-reported, as in the original study. Written, informed consent was given by all participants (see Appendix M-N for details of information sheet and consent form). The 2010 research was given a favourable ethical opinion by the Local Research Ethics Committee (National Health Service 10/H1109/25; Appendix O) and the University of Surrey Research Ethics Committee (EC/2010/53/FHMS; Appendix P). All research was conducted in accordance with the Declaration of Helsinki.

### 2.2.3 Light exposure and sleep health sub-study

#### 2.2.3.1 Study design and protocol

In September and October, a sub-section of the main study participants (n=47) including both postmenopausal and premenopausal women, took part in the sleep and light sub-study. A summary of the background characteristics of the sub-study participants is provided in chapter 5, Section 5.3.1 (n=22 postmenopausal Caucasian, n=5 premenopausal Caucasian, n=13 postmenopausal Asian and n=7 premenopausal Asian). All these participants agreed to wear an Actiwatch (AWL) on their wrist.
for 2 weeks, to measure rest-activity cycles. Of these participants, a further subset (n=32) (n=16 postmenopausal Caucasian, n=4 premenopausal Caucasian, n=9 postmenopausal Asian and n=3 premenopausal Asian) also wore an Actiwatch-L (AWL-L) monitor on a neck cord to measure light exposure. The neck worn monitor was worn in the daytime hours only, but the Actiwatch was worn for a full 24 hours. All participants had completed a Pittsburgh Sleep Quality Index (PSQI) questionnaire, as part of the lifestyle questionnaire for the main study (see Appendix I). In the weeks when they wore the Actiwatches, participants kept a daily sleep diary (see Appendix Q). They also kept an Actiwatch log (see Appendix Q), whereby they recorded all periods when the Actiwatch was removed (e.g. for bathing, washing up). Participants completed the Munich Chronotype Questionnaire once (Roenneberg et al. 2007) (see Appendix R). Participant’s instructions for the use of Actiwatches is shown in Appendix S. See Section 2.3.4 for full justification for methods used in the light exposure and sleep health sub-study. Separate consent was taken for this sub-study (see Appendix T-V for information sheet, study invitation and consent forms). After the sub-study period finished, participants returned the Actiwatches, sleep diaries and actiwatch logs through the post to the University.

The study was scheduled so that the South Asian participants avoided the Ramadan period, when rest-activity cycles change. It was also scheduled so that all participants would be finished before midnight on 30th October 2010 when the clocks converted back from British summer time (BST) to Greenwich Mean Time (GMT). Due to the variation in light levels and weather during different study weeks, wearing of Actiwatches was counterbalanced as far as was possible. This was achieved by allocating an equal number of premenopausal and postmenopausal women and equal numbers from the two ethnic groups, wearing the Actiwatches to the same week. Some small changes to this rota had to be made due to participants only being able to participate in certain weeks, but a good balance was still achieved despite this. The rota for the wearing of the Actiwatches is illustrated in Figure 2.3, with details of final n numbers in each rota.
## Methodology

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Wrist worn Actiwatch rota (n=47)
Rota 1Blue n=13: n=4 postmenopausal Asian; n=9 postmenopausal Caucasian
Rota 2Yellow n=16: n=5 postmenopausal Asian; n=3 premenopausal Asian; n=6 postmenopausal Caucasian; n=2 premenopausal Caucasian
Rota 3Buff n=7: n=2 postmenopausal Asian; n=1 premenopausal Asian; n=3 postmenopausal Caucasian; n=1 premenopausal Caucasian
Rota 4Grey n=11: n=2 postmenopausal Asian; n=3 premenopausal Asian; n=4 postmenopausal Caucasian; n=2 premenopausal Caucasian

Light worn Actiwatch rota (n=35)
Rota 1Blue n=12: n=4 postmenopausal Asian; n=8 postmenopausal Caucasian
Rota 2Yellow n=10: n=3 postmenopausal Asian; n=2 premenopausal Asian; n=4 postmenopausal Caucasian; n=1 premenopausal Caucasian
Rota 3Buff n=6: n=3 postmenopausal Asian; n=2 postmenopausal Caucasian; n=1 premenopausal Caucasian
Rota 4Grey n=7: n=1 postmenopausal Asian; n=2 premenopausal Asian; n=2 postmenopausal Caucasian; n=2 premenopausal Caucasian

Figure 2.3: Rota for wearing Actiwatches
2.2.3.2 Actiwatch ‘neck worn’ monitor light data cleaning and analysis

The light data obtained from the neck worn Actiwatches were analysed using the standard software provided by the manufacturer of the Actiwatches (Cambridge Neurotechnology Ltd). The light analysis program in the Actiwatch software was used to calculate the amount of time spent above five reference lux thresholds (100 lux, 200 lux, 500 lux, 1000 lux and 2000 lux) for each participant over the 14 days. For this analysis the following editing procedure was undertaken.

All AWL units used were calibrated to ensure accurate, comparable light data. The light sensor of each Actiwatch was standardised against a calibrated Powermeter (Maccam Ltd) to ensure comparable light levels. The calibration procedure was as follows. A dark room was set up with thirteen areas of different light intensity, between 10 and 6000 lux (e.g. 15; 45; 110; 250; 360; 450; 570; 660; 770; 1050; 1800; 2500 and 5000 lux). The Powermeter was used to check the accuracy of these intensities at marked positions in the room, in each area of different lux intensity.

Each actiwatch was placed individually in each lux intensity area for 10 mins, with light levels being recorded every 15 seconds, giving 40 values per light intensity, (with the time of each exposure recorded). The mean and SD were calculated for each intensity for each Actiwatch. Graph Pad Prism (v6) was used to fit a second order polynomial (quadratic) curve for the Powermeter reading (y) from the Actiwatch reading (x), with the constant (B0) set as zero. Best fit values were used for the coefficients B1 and B2. The polynomial equation was then used to calculate the x values (Actiwatch readings) that corresponded to five different lux intensities (100 lux, 200 lux, 500 lux, 1000 lux and 2000 lux) (y values) on the Powermeter.

When analysing the light threshold data, these readings were used in place of 100, 200, 500, 1000 and 2000 lux, and varied for each Actiwatch (as each Actiwatch has a different equation). For example, if 100 lux (y) on the Powermeter corresponded (according to the equation) to 166 lux (x) on the Actiwatch, then 166 lux was entered into the light analysis on the Actiwatch software (Cambridge Neurotechnology Ltd), rather than 100 lux itself. This ‘corrected values’ procedure was repeated for all the actiwatch lux thresholds for each participant’s data.

This light analysis procedure produced estimates for minimum number of minutes spent above each of the five lux thresholds, for each participant, for each day of the study period (14 days). These data were copied to Microsoft Excel. Any days where compliance with the wearing of the neck monitor was problematic (i.e. there was no movement seen on the Actogram for the neck monitor all day, or there was a period of no movement for more than 3 hours) were completely discarded from the analysis. Therefore, some participants had less than 14 days of data remaining. Any participants
with less than 7 days remaining had all their data removed from the analysis, as it not deemed valid to have less than 7 days of data. Last, data were inspected for the presence of abnormal results (values outside 3 SD of the mean were examined for all participants). There were no abnormal results, so no further data needed to be excluded.

Daily time profiles were also produced for each participant, showing mean median lux for each participant for each hour of the day. Prior to conducting this time profile analysis, it was crucial that the zeros in the light data, for the day time period only (7:00 h to 24:00 h) were removed. This is because zero values during the day are ‘artificial zeros’ in that they are due to accidental coverage of the monitor by clothing. These artificial zeros can skew the data. Conversely, zeros did not need to be removed at night as these are likely to be legitimate zero values (as the environment is likely to be very dark). The period where zeros were not removed was deemed ‘night time’ (24:00 h to 07:00 h) as it was the latest time to bed, and earliest wake time of all our participants during the study period. The zero values were removed using a macro (created by Peter Williams, University of Surrey) in SAS software (SAS Institute, Cary, NC, USA), such that if in an hour period, there were 10 zeros and 50 non zero values, the code removed the 10 zeros, and divided the remaining values by 50, thus stopping the removal of zeros skewing the data.

This macro then calculated the median lux levels for each hour. Median lux was used as mean lux values are known to be misleading, due to the possible skewed nature of light data. This occurs due to the occurrence of short bursts of high intensity light if outside on a sunny day. The median hourly lux values were copied into Microsoft Excel. As above, any days where participants had not worn the Actiwatch at all, or had a gap of more than 3 hours, were discarded from the analysis. Also, any participants with less than 7 days of valid data were discarded completely from the analysis. The mean median lux was calculated for each remaining participant for each hour of the day, over the 14 day period.

These mean median lux values were corrected for accuracy of the light sensor, as were the light threshold data, albeit by a slightly different procedure. The procedure here was to use an excel spread sheet to correct the mean values, using the polynomial equation specific to each Actiwatch. Next, participant data were divided up into ethnic-menopausal status groups, and an overall group mean median lux was calculated for each hour of the day. Data were inspected for the presence of abnormal values (values outside 3 SD of the mean were examined for all participants).No abnormal data was found, so no further data needed to be excluded. Finally, time profiles were produced, with light intensity at each hour of the day plotted (e.g. at 00:30 h, 01:30 h etc. up to 23:30 h) for both individuals and groups.
2.2.3.3 Actiwatch ‘wrist worn’ activity data cleaning

Actiwatches were set to record data every minute, over the whole 24 hours, over 14 days. A 14 day period was chosen because there is a large day to day variability within subjects for Actiwatch readings (Knutson et al. 2007) and the reliability of the Actiwatch recordings are higher if a longer time duration is used (Van Someren 2007). A duration of at least 5-6 days is advised for adequate reliability in middle aged adults (Knutson et al. 2007). Before analysis, the Actiwatch data for the wrist worn watches needed to be edited to ensure valid results. This was mainly to remove periods of time when the watch was not being worn by the participant, or rarely, where data artefacts (activity spikes) had been produced by the actiwatch. The procedure for each watch was as follows. The period where the participant said the Actiwatch was off (from records) were located. Using Microsoft Excel, the exact time this occurred was scanned for and noted. This time period was replaced with the mean activity value for that day (as calculated by the sleep software programme). Only time slots over ten minutes were changed. For recorded blank time slots over 3 hours, nothing was changed but it was noted down that this day needed removal from analysis.

Then, the data were scanned for any unrecorded large time slots (over 1 hr in the day- which is enough to skew the data). Any one hour or greater slot was replaced with the daily mean. As with recorded slots, unrecorded slots of over 3 hours duration led to the day being discarded from the analysis. For night readings, the data was checked to ensure that the participant had worn their Actiwatch all night long (i.e. no very long gaps of longer than 90mins without movement at all). If this did occur the whole day was excluded from analysis. If it was only a gap during the daytime (07:00 h-24:00 h) then it was deemed valid for the sleep analysis and the day kept in. Nights were excluded from the sleep analysis if the next day had a gap in the early hours of the morning (i.e. days were kept in whenever possible if night or early morning data were intact). Days were only removed if a long gap of >90mins appeared on either night after bedtime or early morning before wake up). Exclusions were kept to a minimum. For example, participants with a 2 hour gap in the night, where it was unlikely due to the timing (often 1-2am) that they would have removed the Actiwatch, were assumed to be displaying a true sleep. However only gaps less than or equal to 2 hours were accepted for this. Any data spikes (i.e. activity > 5000) were scanned for and if in daytime hours were replaced with the daily mean value.

2.2.3.4 Actiwatch sleep analysis

As with the light data, the activity data were analysed using the bespoke sleep analysis software (Cambridge Neurotechnology Ltd). Each participant’s data (n=47) were run separately by the software, using the following procedure. From the sleep diary data, the earliest time to bed, and the
latest wake up time were identified for each 14 day diary. These times were entered into the software to signal to the program where the sleep period was likely to be for the participant (i.e. the analysis ‘window’). The actual time to bed (i.e. try to sleep) and get up time (i.e. wake time) for each individual day for each of the 14 days was also identified and entered into the software, to show the program exactly where the participant reported trying to sleep at night and waking up the next morning. The sleep analysis program was then run for the full 14 days that the participant had worn it, and the procedure repeated for all participants.

This data was exported to Microsoft Excel, with parameters that had clock times as outputs being converted to decimal hours. This conversion was undertaken as most software programs cannot use clock time formats. The means for each participant were recalculated after the removal of any days with unreliable data. Then, all data for participants who had less than 7 remaining days of data were removed (n=5). The recalculated mean and SD for the remaining participants (n=42), were then used for statistical analysis. Data were inspected for the presence of outliers (values outside 3 SD of the mean for all participants). There were very few outliers, according to this definition, so no data were excluded.

2.2.3.5 Non-parametric circadian rhythm analysis (NPCRA)

NPCRA was also run for each participant’s wrist worn actiwatch, for all 14 days, using a Microsoft Excel Macro (devised by E.Van Someren). The NPCRA macro was asked to ignore days that were not deemed valid, in accordance with the data cleaning rules. For discarding of data the same rules were applied as were used for the activity analysis (see Section 2.2.3.3 for full description of rules). The exception to this was that days were always removed if there was a 3 hour gap in any of the day’s data, unlike in the other sleep analysis whereby days could be retained if the gap was not affecting the sleep period. This is because NPCRA assesses both the sleep and non-sleep periods, unlike the other sleep analysis which only examined the sleep period. The NPCRA enabled estimation of the sleep parameters; L5, M10, L5 onset, M10 onset, amplitude, inter-daily stability and intra-daily variability (see Chapter 5, Section 5.2.3.1 for definitions of these parameters). Data were inspected for the presence of abnormal values (data points outside 3 SD of the mean were examined for all participants). There were no abnormal values, so no further data were excluded.

2.2.4 Interview sub-study

From February to June 2011, semi-structured, qualitative interviews of around one hour were undertaken with a subset (n=28) of the older postmenopausal participants. Details of the background characteristics of the interview participants can be seen in Chapter 5 (Section 5.2). As with the light
exposure and sleep sub-study, at the main study visit the participants were given the option to take part in the interview sub-study. The interview sub-study was restricted, however, to the postmenopausal women, aged 60-70 years, only. This was to enable a more homogenous study group for the analysis. Refer to Figure 2.2 (Section 2.2.1) for flow of participants into the interview sub-study. Justification for the use of interview technique, including the semi-structured format, can be found in Section 2.3.8. See Appendix W for the interview schedule.

Materials used for the interviews included a standard Sony (TCM939) analogue tape recorder with built in microphone. Standard blank cassette tapes were used for recording, and labelled with participant number only, for reasons of confidentiality. The interview questions were printed on paper, which was kept by the interviewer at all times on a clipboard. Interviews were conducted in private rooms within the University of Surrey, or in the Asian Women’s network buildings. Refreshments were provided during the interviews for both participants and interviewer. The layout of the room was made to be as informal as possible (e.g. rearrangement of chairs) to enable the participant to feel relaxed. Consent was taken for the interview sub-study on the day of the interview. The interviewees had been provided with an information sheet (Appendix T), study invitation (Appendix U) and consent form (Appendix V) for viewing prior to the interview taking place. The interview data transcripts were analysed using qualitative analysis software NVivo8.0 (QSR International Ltd, Australia).

2.3 Explanation and justification for methods used

2.3.1 Assessment of vitamin D status- serum 25(OH) D

The serum metabolite 25(OH)D is the most widely used measure of vitamin D status. This is due to the fact that 1,25(OH)₂D, the active metabolite of vitamin D, is tightly controlled metabolically. Thus, when an individual is deficient in vitamin D, 1,25(OH)₂D may be only slightly below normal. However, in deficient individuals, the precursor molecule, 25(OH)D, is usually low.

Despite universal agreement in 25(OH)D being the most valid measure of vitamin D status, there is much disagreement as to which assay should be adopted (Lai et al. 2010), due to large inter-assay variation. To compound this problem, there is also an issue about large variation in results between laboratories (Lai et al. 2010). Fortunately, results from the last two cycles of DEQAS (Vitamin D quality assurance scheme) suggest this problem is reducing, with inter-laboratory and inter-assay precision improving (Carter et al. 2010). 25(OH)D for the DFINES study (2006-2007) was measured at the University of Manchester by the team of Dr Jacqueline Berry, using the manual
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Immunodiagnostic Systems (IDS) enzyme immunoassay method (Boldon, UK). In terms of quality control, the IDS enzyme immunoassay method has given only a 3-5% positive bias over the all laboratory trimmed mean (ALTM) in two recent DEQAS cycles (2007-8 and 2008-9) (Carter et al. 2010). However, the main disadvantages of assay kits such as the IDS enzyme immunoassay are that D2 and D3 cannot be differentiated, only a total 25(OH)D concentration can be given. This is unlike when HPLC and LC-MS/MS are used, whereby measurement can be made of the relative quantities of both metabolites (reviewed by Lai et al. 2010). Indeed, HPLC is known to be the gold standard for measurement of vitamin D status.

Our choice for analysis of 25(OH)D in 2010 was restricted by the fact that the original study used the IDS immunoassay. In order to validate our longitudinal analysis between 2006 and 2010, it was necessary to replicate the original method used in 2006. Thus, the same assay method and laboratory was used in 2010 as was used in 2006. HPLC was also used in 2010, in addition to the immunoassay kits. The reason for this was so that the gold standard method was available for use in analyses where a comparison with the 2006 data was not required.

2.3.2 Bone markers (sCTX)

Bone is continually being formed and broken down at areas of the bone called Bone Remodelling Units (BMU)s. Osteoclast cells resorb the bone, which is subsequently filled in with new bone material by osteoblast cells. Bone markers are a quick and non-invasive way of obtaining information about this bone turnover process. They are usually either enzymes produced by the cells controlling bone turnover, or are the waste products of bone breakdown from this process.

The bone marker measured in the D-FINES study 2006-2007 was serum C-terminal cross-linked telopeptide (sCTX). Serum CTX is a reliable marker of bone resorption, being both specific and sensitive (reviewed by Eastell and Hannon 2008). It is a breakdown product of collagen degradation from the bone matrix, thus its concentration in blood or urine is a measure of bone resorption. For that reason, high levels indicate increased bone resorption activity, which if prolonged and not coupled with concurrent increased bone formation, is indicative of current or future poor bone health. In females, sCTX concentrations increase with age (Filip and Zagorski 2004). Evidence shows high levels of sCTX to be associated with increased bone loss (Garnero et al. 1999) and to be predictive of future fracture (Chapurlat et al. 2000). The benefit of serum CTX is that, unlike urine CTX, (or urine n-telopeptide of collagen; uNTX) it can be measured without needing the concurrent measurement of creatinine.
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The known reliability and validity of sCTX for measuring bone resorption, as well as cost and availability considerations, meant it was the choice of measurement for bone resorption in D-FINES 2006-2007. Alternative bone resorption markers that could also have been chosen for the D-FINES study included the other collagen degradation products such as hydroxyproline, pyridinoline and deoxypyridinoline. However, like sCTX, hydroxyproline and deoxypyridinoline measures can be problematic as they are not specific to bone collagen. Hydroxyproline is also influenced by dietary intake of gelatin. Pyridinoline has the benefit of being specific to bone and dentin, however this bone marker is reasonably expensive, so was not able to be used in the D-FINES study due to cost considerations.

As described in Section 2.1.3, samples in the D-FINES (2006-2007) study were collected fasted, and at the same time of day (between 8am and 11 am). This was to prevent confounding by diurnal variation, (as recommended by Brown et al. (2009)). Despite the positive aspects described above, it must be borne in mind that there are limitations to using bone markers to assess bone health. First, as with all biological analytes, there is an issue with variability in measurements. This is a concern for the usage and interpretation of bone markers (Vesper et al. 2004). For this reason, in the DFINES 2006-2007 study, all the seasons’ sCTX measurements were analysed together in the same batch, in the same laboratory, at the same time, to reduce the confounding effect of variability. Second, there are known lifestyle (diet and exercise) as well as seasonal and hormonal effects on bone remodelling which must also be considered when interpreting results (Vesper et al. 2004). Thus, there is a concern over intra-individual variability in results. Last, bone markers cannot give direct information about bone geometry or properties of the bone mineral. They can only give an indication of the current degree of bone remodelling that is occurring. Other methods such as pQCT, HR-pQCT, BUA or DXA are required to assess longer term structural changes in bone health. For this reason, pQCT was used in the main summer 2010 study to give information about bone geometry.

2.3.3 pQCT

It is important when assessing bone strength that both the quality of bone and its structure are considered. As can be seen in Figure 2.4, bone strength relies on bone size and the thickness of the cortex, as well as on bone density. The strongest bones are both dense, large in cross sectional area and have a relatively thick cortex.
Figure 2.4: Impact of bone geometry on bone strength

pQCT is a technique that is used to assess bone structure and strength parameters at the radius and tibia. It gives much more detailed information than bone density alone. The technique uses x-rays to image slices through the radial or tibial cross section, in order to calculate size, mass and density parameters, including that of the cortical and trabecular layers separately. See Figure 2.5 for examples of the images produced using pQCT at the distal radius and tibia.

Figure 2.5: pQCT image of a slice through the distal radius (left) and distal tibia (right).

A schematic of some of the geometric parameters obtained from pQCT in relation to distal radius can be seen in Figure 2.6. From these measurements, the software calculates predicted fracture load (amount of Newtons required to break the bone) and strength strain indexes (SSI; polar and axial). Equations used by the pQCT software are shown in chapter 4 (Section 4.2.2).
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Figure 2.6: A schematic of some of the geometric parameters obtained from pQCT at the radius

See Figure 2.7 for an illustration of typical pQCT parameters measured at the radius and tibia. pQCT is superior to DXA as it allows examination of the geometric properties of bone, including the density of both types of bone (cortical and trabecular). In addition, it allows calculation of strength parameters (e.g. torsion, stress-strain index, polar moments of inertia). DXA can only measure aBMD and bone size so it cannot give detail about the densities of the different parts of the bone. vBMD is a better estimate of true bone density, as the measurement is not confounded by bone size. This means pQCT is particularly useful for assessing ethnic differences in bone health, whereby it is important that data are not confounded by bone size.

The two main limitations of using DXA are bone size and the inability to differentiate between different bone types (Hollis et al. 2007). pQCT overcomes both of these limitations. pQCT is also superior to QUS, which, like DXA, is also confounded by bone size. Also, like DXA, QUS does not give as detailed information about bone micro architecture, geometry and strength. However, pQCT does have some limitations. First, it is mainly a research tool, as it has not been found clinically to add any extra predictive ability to fracture prediction over information from DXA alone (Black et al. 2008). Also, it cannot assess bone health at the hip and spine (central sites), which are the two most significant sites for osteoporotic fracture. It can, however, measure the distal radial site, which is of some importance for osteoporotic fracture and it has been found to be predictive of non-vertebral fractures (Sheu et al. 2011).
Figure 2.7: A schematic of the parameters measured using a variety of pQCT radial and tibial scan sites

For the purposes of our study, which is to obtain information on bone structure in the two ethnic groups, pQCT is particularly useful as measurements at the radius and tibia can give us important scientific information as to the differences in bone structure and strength by ethnicity, and differences in the underlying biological mechanisms affecting skeletal health in different ethnic groups.

The other disadvantages of standard resolution pQCT include the fact that the resolution is too low to clearly assess micro-detail of the trabeculae. High resolution peripheral computed tomography (HR-pQCT) can image the trabeculae, including the number of rods and plates, and the connectivity between them (see Figure 2.8 for an example image from HR-pQCT).

Figure 2.8: HR-pQCT slice through the distal radius (L) and tibia (R) showing detail of trabeculae
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Also, standard resolution pQCT cannot inform about the properties of the bone mineral material (e.g. brittleness, micro-cracks). These properties can only be estimated in vivo using HR-pQCT (or in vitro using examination of cadavers). HR-pQCT can enable finite element modelling, a statistical modelling technique which can visualise the predicted behaviour of the bone under strain and the conditions under which failure is more or less likely. HR-pQCT is a more detailed and informative method for assessment of bone structure and strength than standard resolution pQCT. However, it is more expensive and involves a higher radiation dose per scan (3 microsieverts for a HR-pQCT scan (MacNeil and Boyd 2008) vs 1 microsievert for a pQCT scan (STRATEC 2008). HR-pQCT shares the disadvantages of pQCT in terms of measuring only peripheral sites.

To summarise, HR-pQCT would have been the method of choice, but as HR-pQCT was not available, the use of standard resolution pQCT in the 2010 study allowed information to be obtained on bone strength, true volumetric density and bone geometry. It enabled a true assessment of volumetric bone density, not confounded by bone size. This was important in order to answer the question as to whether Asian women do or do not have ‘weaker’ bones than Caucasian women.

2.3.4 Actigraphy, PSQI and sleep diary

Actigraphy is a validated tool for assessing rest-activity patterns. It consists of a watch like device (Figure 2.9) which contains an accelerometer.

![Figure 2.9: Photograph of Actiwatch-L monitor](http://www.jdinstruments.com/actiwatchl.html)

It measures wrist movement at set intervals (‘epochs’), usually of 1 minute. Based on the principle that when people are asleep, their body is mostly still, specialised software is used to calculate rest-activity parameters. It achieves this using the amount of time day and night, that the wrist is moving, as well as the degree of movement. This activity data is presented as an Actogram (Figure 2.10) and
Figure 2.10: Example Actograms for Light exposure and Activity (1 week’s data) from an Actiwatch-L
parameters such as sleep duration, sleep latency, sleep efficiency and sleep fragmentation can be estimated. In addition, Actiwatch-L models also contain a lux meter, which is able to measure light exposure. An equivalent Actogram can also be produced for the light readings (Figure 2.10), when using the Actiwatch-L device.

The full list of parameters available using Actiwatch data are listed in Table 2.2. The advantages of Actiwatch use in sleep research are as follows. First, they have the benefit, unlike polysomnography, of being able to be conducted by the participant in their own homes, which may give a more realistic idea of their usual rest-activity cycles. Studies have shown that actigraphy shows good correlation with measurements by polysomnography (reviewed by Sadeh and Acebo 2002). Second, Actiwatches are relatively non-invasive and require reasonably little work by the participant. However, there are some disadvantages to the use of actigraphy. First, Actiwatches are not waterproof, so they must be removed during certain tasks (e.g. bathing and swimming). Therefore, the participants need to note times when the watch is off and then the data from these time periods must be removed from the analysis by the investigator. Second, compliance is often a problem, with participants not wearing the watch as instructed. If this is long enough to cause distortion in the data, periods of non-compliance need to be removed from the dataset.

Many of the compliance problems with Actiwatches can be overcome by giving participants clear instructions, and by monitoring them by telephone. Asking participants to keep a sleep diary in conjunction with actigraphy is useful in interpreting the Actigraphic results, as it enables clarification of the participants’ self-reported sleep period and periods when the device was not worn. Last, and perhaps most importantly, as Actiwatches use arm movement to assess activity levels, they are not completely accurate in allowing estimation of whether the person is asleep or not. This is due to the fact that even when awake a person’s arm can be completely still (e.g. when reading in bed, or watching television). Actigraphy only gives an indicator of rest-activity patterns, from which time asleep and awake can be inferred. Hence, actigraphy has been found to be less useful if precise estimates of sleep wake cycles are required (reviewed by Sadeh and Acebo 2002).

The Actiwatch-L (rather than the original Actiwatch) was chosen for this study as it has the benefit of also being able to measure light exposure. As well as wearing an Actiwatch-L on the wrist, willing subjects were also asked to wear a second Actiwatch-L around their neck. This was to enable a better estimate of true light exposure. This was because neck worn Actiwatch-L monitors are less likely to be obscured by clothing, as well as being closer to the eye, so giving a more realistic measure of the light that the eye receives, rather than just ambient light levels.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time ‘try to sleep’</td>
<td>Time that the participant tried to sleep (i.e. lights off, laying still)</td>
</tr>
<tr>
<td>Wake time</td>
<td>Time that the participant woke up fully in the morning (eyes open)</td>
</tr>
<tr>
<td>Time in bed</td>
<td>The difference between time ‘try to sleep’ and wake time</td>
</tr>
<tr>
<td>Sleep start</td>
<td>Start of sleep as defined by operator or sleep algorithm</td>
</tr>
<tr>
<td>Sleep end</td>
<td>End of sleep as defined by operator or sleep algorithm</td>
</tr>
<tr>
<td>Assumed sleep time</td>
<td>Difference between sleep start and sleep end</td>
</tr>
<tr>
<td>Actual sleep time</td>
<td>The amount of sleep as determined by the software algorithm</td>
</tr>
<tr>
<td>Actual wake time</td>
<td>The amount of time awake as determined by the software algorithm</td>
</tr>
<tr>
<td>Sleep efficiency</td>
<td>Actual sleep time/time in bed</td>
</tr>
<tr>
<td>Sleep latency</td>
<td>The amount of time from time ‘try to sleep’ to sleep onset</td>
</tr>
<tr>
<td>No. of sleep bouts</td>
<td>The actual number of episodes of sleep</td>
</tr>
<tr>
<td>No. of wake bouts</td>
<td>The actual number of bouts of wakefulness</td>
</tr>
<tr>
<td>Mean length of sleep bouts</td>
<td>Total duration of sleep bouts divided by number of sleep bouts</td>
</tr>
<tr>
<td>Mean length of wake bouts</td>
<td>Total duration of wake bouts divided by number of wake bouts</td>
</tr>
<tr>
<td>No. of mins immobile</td>
<td>Total number of minutes during assumed sleep period when activity counts per minute are below a predetermined threshold</td>
</tr>
<tr>
<td>No. of mins moving</td>
<td>Total number of minutes during assumed sleep period when activity counts per minute are above a predetermined threshold</td>
</tr>
<tr>
<td>% of mins immobile</td>
<td>Number of minutes immobile / assumed sleep period</td>
</tr>
<tr>
<td>% of mins moving</td>
<td>Number of minutes moving / assumed sleep period</td>
</tr>
<tr>
<td>No. of immobile phases</td>
<td>The number of periods made up of consecutive epochs when the counts are less than the immobility threshold</td>
</tr>
<tr>
<td>No. of immobile phases of 1 min</td>
<td>The number of immobile phases where the duration is no longer than one minute</td>
</tr>
<tr>
<td>% immobility</td>
<td>The number of immobile phases of one minute as a proportion of the number of immobile phases</td>
</tr>
<tr>
<td>Fragmentation Index</td>
<td>The addition of % minutes moving and % immobility</td>
</tr>
</tbody>
</table>

* operator entered from sleep diary; ¥ not necessarily the time they got out of bed
For this reason, light data collected by neck worn monitors, not wrist worn, was analysed. The wrist worn watch was solely used to measure activity levels.

The Pittsburgh Sleep Quality Index (PSQI) was used in this study to measure self-reported sleep quality. The PSQI is a well validated questionnaire which has been found to give reliable results in distinguishing people with a sleep disorder from those who do not (Buysse et al. 1989). See Appendix I for details of the PSQI (within lifestyle questionnaire) and Appendix X for information regarding the scoring algorithm. The structure of the PSQI with its component subscales are listed in Table 2.3.

Table 2.3: Structure of the PSQI

<table>
<thead>
<tr>
<th>Sub scale</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep duration (DURAT)</td>
<td>Score 0-3</td>
</tr>
<tr>
<td>Sleep disturbances (DISTB)</td>
<td>Score 0-3</td>
</tr>
<tr>
<td>Sleep latency (SOL)</td>
<td>Score 0-3</td>
</tr>
<tr>
<td>Daytime dysfunction (DAYDYS)</td>
<td>Score 0-3</td>
</tr>
<tr>
<td>Habitual sleep efficiency (HSE)</td>
<td>Score 0-3</td>
</tr>
<tr>
<td>Sleep quality (SLPQUAL)</td>
<td>Score 0-3</td>
</tr>
<tr>
<td>Sleep medications (MEDS)</td>
<td>Score 0-3</td>
</tr>
<tr>
<td>Global score *</td>
<td>0-21</td>
</tr>
</tbody>
</table>

*as per algorithm (see Appendix X)

2.3.5 Bone pain questionnaire

It is known to be difficult to assess people’s experience of pain, due to its subjective nature, and the difficulty people have in describing it (Strong et al. 2009). In order to assess prevalence of musculoskeletal pain it was imperative that adequate methods were produced. Some studies have used clinical examination by a doctor (as in small patient studies and case reports). However, in this study, it would have been unfeasible for participants to have undergone a clinical examination by a doctor meaning that self-report was the only viable option.

A search of the literature revealed no validated or commonly used questionnaire to assess musculoskeletal pain, and certainly not one that was tailored to also distinguish vitamin D deficiency pain from that of other pain types. Most research studies have used ratings questionnaires or simply ask if widespread pain is present or not. In non-clinical studies, researchers have used short questionnaire methods, with visual analogue scales (Likert type ratings) to assess musculoskeletal pain. These questions may be supplemented with body pictures (‘manikins’) or by ‘grid type’ questions (Macfarlane et al. 2005; Atherton et al. 2009; Parsons et al. 2006).
Based on the methods used in other studies a questionnaire was developed. The use of pain manikins (body shaped diagrams where participants indicate the location of pain) was considered (Figure 2.11). However, this idea was rejected as it was deemed too confusing for the participant to mark both location and severity of pain at once. One study had discussed the benefits of manikins for poor English speakers and those of different cultures (van den Hoven, Gorter, and Picavet 2009) but interestingly, in their final results, little difference was found between pain ratings given by manikins versus answering questions.

Figure 2.11: Example of a ‘pain manikin’

The grid type of questions was also considered. One study showed that people filled in grid type questions well, even when many different body areas were assessed, with good reliability and validity (Parsons et al. 2006). Parsons et al. (2006) also stated that this method may be most useful when people do not have a diagnosis, just symptoms, and that hip and thigh ratings corresponded well to the results gained from manikins. Therefore, this method was deemed a good one for use in our non-patient population, who had symptoms, but not a diagnosis, of bone and muscle pain.

It was decided that a grid table may be most straightforward for participants to understand, with body site on rows, and severity in columns. Participant could tick the appropriate box in each row and it was thought that this would encourage them to think through all the parts of the body in turn, giving a more comprehensive view. It was believed by the study’s investigators that the manikins may encourage the choice of one site only (even if pain was widespread throughout different areas), although this has not been explicitly assessed in the literature.

The most important aspect for the current study was to have pain ratings for severity, pain type and location of the pain. This was important for understanding if any pain reported was likely to be vitamin D deficiency pain or not. As used in the McGill pain questionnaire (Melzack 1975), we included examples of the type of pain, including throbbing, shooting, dull, aching, burning etc. As
can be seen in Appendix F, Q24 to 25, and Q28 to 29 on the study questionnaire were designed to assess whether the thigh pain weakness were likely due to vitamin D deficiency, and Q26 to assess whether joint problems were part of the cause of their pain.

Questions 4 to 14 on the questionnaire related to back pain. These questions were included to enable assessment as to whether this was likely to be a factor in the answers to the other questions asking about severity, location, frequency, pain type, and use of pain relief for back pain. Questions 15 to 22 examined the ability of participants to perform tasks of daily living. The questions about back pain and the tasks of daily living scale were adapted from the Back Pain and Function scale used in the National Institute of Health (NIH) Study of Osteoporotic Fractures (NIH Study of Osteoporotic Fractures, 2003) (N.I.H. 2003). The questions on this scale were Likert type, whereby a response is chosen from 0 (no difficulty) to 3 (unable to do) (see Appendix F for question details). As in the Study of Osteoporotic Fractures, it was planned that the scores from this scale (Q15 to 22) would be summed to give an overall score for tasks of daily living. Question 23 (bone pain; Figure 2.12) was one of the two grid-type questions (the other question was question 27; muscle pain), whereby responses were given for each area of the body, and a visual analogue scale given to assess pain severity. In accordance with good practice for questionnaire design, questions were routed so that participants were directed to questions that matched the answers they had given. For example, if they said they had no back pain, they were asked to go to the next section, rather than being asked more questions about back pain.

23. (a) Have you had any pain in your bones?
   Yes?  No? (go to question 25)
   (b) On the table below, please tick the boxes for any areas where you have had bone pain and then rate the severity of the pain on the horizontal line (where 1 = no pain and 10 = severe pain):

   ![Bone Pain Questionnaire](image)

   Figure 2.12: Question 23 from the bone pain questionnaire
2.3.6 Grip strength, stand to walk tests and other questionnaires

In the D-FINES 2006 study, grip strength was used as a measure of musculoskeletal strength. Being simple to use, and quick to carry out, it was used again in 2010, using the same procedures and equipment (JAMAR hydraulic hand dynamometer; see Figure 2.13 for an illustration). Measurements of strength from hand held dynamometer devices have been found to be comparable to that from isokinetic muscle testing (Stark et al. 2011). However, due to the known benefits of assessing more than one body area and muscle group, when testing strength (Bohannon 2008), the stand to walk test was also used. This test requires timing the amount of time it takes a person to rise from a chair and walk a set short distance (e.g. 3m; 5 times), and has good test-test reliability (Jette et al. 1999). Also, it takes into account thigh strength, which is useful in that it is the thigh muscles being weak that contribute to vitamin D deficiency mobility problems, including the characteristic ‘waddling gait’.

For the health, lifestyle and physical activity questionnaires, the same questions and format were used in the 2010 study as had been used in the 2006-2007 study. Despite the limitations of some of the questions in the original study, it was deemed validity of the longitudinal analysis was of most importance. This required that the wording of the questions remained unchanged. The PSQI (see Section 2.3.4) was added to the lifestyle questionnaire to limit the number of separate questionnaires handed out. All questionnaires used in the 2010 study can be seen in Appendices F-K.


Figure 2.13: The JAMAR hydraulic hand dynamometer

2.3.7 Diet Diary and Dosimeter Badges

The gold standard for diet diary analysis is the seven day weighted food record. However, this method requires food weighing, so it was felt that this would be likely to be burdensome for the participants. Also, it was important to replicate the method used in 2006-2007 to enable valid longitudinal comparisons. Hence, as with the original D-FINES study, a four day (including a
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weekend day) food diary with photographs to aid portion estimation was used. This was a validated diary used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. This type of estimated food diary has been found to give comparable results to a weighed food record (Bingham et al. 1994). There were also no significant practical issues with the use of the diaries in the original study. However, it must be borne in mind that vitamin D intakes may be underestimated with all short term recording methods, due to some good sources of vitamin D commonly being eaten infrequently (e.g. oily fish).

Dosimetry is a common technique used in research studies to assess UVB exposure. It involves the participant wearing a polysulphone film badge on the exterior of the clothing when outdoors. This type of dosimetry has been found to be reliable for measuring UVB sun exposure in most situations (Diffey 1987). There is no other type of dosimeter available at present for measurement of UVB exposure. The polysulphone dosimeter badges were used successfully in the original DFINES study, giving more reliable and consistent results than the sun exposure diaries, which were subject to poorer participant compliance. The sun exposure diaries were not used in the 2010 study due to these issues. As with dietary analysis, it was necessary to repeat the previous method for quantifying sun exposure (dosimeter badges) to enable a valid longitudinal analysis.

2.3.8 Interviews

Qualitative data analysis is becoming increasingly used in health care research. This type of data analysis allows an insight into the perceptions, beliefs, attitudes, and experiences of persons with regards to their health, which can complement the more traditional quantitative methods used in this field. This Thesis used the qualitative approach for the analysis of social influences on sun exposure as this approach yields rich, socially relevant information. It is likely to give a much richer and more detailed picture of people's sun exposure behaviour than quantitative survey methods looking at measures of outdoor activity and actual UVB exposure.

2.3.8.1 Types of qualitative research

The main four types of qualitative research include discourse analysis, ethnographic observation, focus groups and interviews. Discourse analysis consists of an in depth analysis of available documentation. Such documentation can include historical accounts, information (e.g. policy documents), current or past media (text, auditory, visual), stories or plays. Discourse analysis is useful in its use of already written texts, making data collection a relatively a small task in comparison to other approaches. However, it is restricted in that the researcher can only use what is
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there, and usually cannot question the author of the works directly. As a result, it was not deemed suitable for answering the Thesis question.

Ethnographic observation involves the researcher immersing themselves in the actual day to day lives of the people being studied and making detailed notes (verbal, auditory or visual) about their actions and behaviours. Researchers may be overt (the participants know they are being observed) or covert (participants are unaware). Researchers may be actively involved in the setting they are observing (e.g. actually interacting with the participants) or just passively observing (i.e. without any interaction). Ethnography, although giving rich data about interactions between people, and how their behaviours are shaped by others, cannot give rich detail about the inner lives of the participants. It is also time consuming to conduct, and may not be possible to conduct ethically or practically in many settings. There would not have been enough time to conduct ethnography in the context of this PhD project, as it involves months of fieldwork, and this Thesis only aims to present a relatively short examination of social factors with a small number of people. Also, the aim of this Thesis is to gain an insight into the attitudes and perceptions about sun exposure, which would be difficult with an ethnographic study.

Focus groups are a useful way of getting people to talk about their opinions and experiences. They involve having groups of participants together at the same time, for a ‘group interview’ or joint task. The researcher records the spoken opinions and comments and experiences produced by each member of the group. Focus groups are useful as they allow group dynamics to be studied against individual attitudes and behaviours. This was considered as a possible methodology for the social section of this Thesis. However, it can be difficult to get people together at the same time and place, especially if the women being studied originate from different geographical areas. Practical considerations such as the preference of the South Asian women to take part at their own centres, whilst the Caucasians were more willing to participate at the university also precluded the use of focus groups for this study. Focus groups may also have been problematic due to staff limitations with only one researcher running this study whilst focus groups are best run with two people; one to guide the participants and the other to monitor the audio recordings and make field notes. Consequently, it was deemed that individual interviews would be the method of choice for this Thesis. Like focus groups, they enable examination of the attitudes, beliefs, perceptions and reported behaviours of the participants, whilst being tailored to the location and time that was most suitable for the participants.

One hour was allowed for each interview with actual time being slightly more or slightly less than this depending on the motivation, or ability of the participant to continue. Ideally, more than an hour is allowed for a single person interview, but time constraints precluded the use of longer sessions in
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the current study. In particular, given the relatively small nature of this sub-study, a need to limit transcription time rather than interview time per se was recognised (it takes 6 hours to transcribe a 60 minute interview). The possible issue of fatigue in the South Asian ladies when expressing themselves in their second language, for more than one hour was also recognised.

2.3.8.2 Qualitative interviews

Qualitative interviews come in three main forms. The first is a structured interview, whereby the interviewee answers very specific questions, and the interviewer cannot deviate from these. The second is a semi structured approach where there is a broad set of questions, but the participant and interviewer are able to move away from the set list as they see fit, whilst keeping in with the broad themes of the interview. An unstructured interview, as the name suggests, has few questions and is a broad discussion on a theme. The latter two approaches are ‘participant centred’ or ‘directed’ approaches, as opposed to the first which is a researcher-directed approach.

For this Thesis, a semi-structured interview approach was chosen. This was deemed the best choice for this research as it allows the participants full scope to be able to freely talk about the issues that affect them and their experiences within the bounds of the questions the interviewer wants to discuss. Sun exposure may be something most people don’t often think about, and the use of open, semi-structured questions may help trigger participant discussion about their experiences.

The main advantage of the semi-structured approach is that it is believed to produce richer and more useful data than highly structured questions. The semi-structured approach may be more useful that the unstructured approach as the interviewer has more control and direction over the content of the interview. This gives more consistency between interviews, as the participants are being asked the same questions.

The main disadvantage of all qualitative approaches is the time consuming and labour intensive process of transcribing the interviews into document form and analysing them, which requires coding each extract of each interview. Recent advances in the development of qualitative analysis software (Computer Aided Qualitative Data Analysis; CAQDAS) have streamlined the analysis process for qualitative research but it remains a time intensive task. Nonetheless, the detailed information that qualitative research provides is deemed worth the large investment in time and effort, especially if transcription services can be used to free up some of the researchers’ time.

In terms of data analysis, a flexible strategy can be used, in that interview conduction, transcription and analysis is not necessarily a linear process. The researcher can combine running interviews with
transcribing and analysing previous interviews in a similar time frame. Insights gained from previous interview analysis can be used to guide and refine the process of conducting and analysis the next interviews in the set.

For the interviews conducted for this Thesis, subjects were recruited on an opportunistic basis from the D-FINES study cohorts. This is quite usual for qualitative research, as unlike quantitative research, recruitment is not usually a random sample. Typically, subjects are recruited by an unsystematic method, with some discretion used by the researcher to ensure either sufficient uniformity, or a variety of participants. A set of participants with similar characteristics allows increased applicability of the results to that subset of people, but not to others. Use of a wider variety of subjects increases generalisation to more people to some extent, but may be too wide to be meaningful about any particular group, or perhaps to everyone at large. Hence, it makes sense with qualitative research to aim for a broadly similar group of individuals, but with enough variety for there to be enough differences to show up nuances, which help build the analysis. However, the aim is to not have so much variety that it is not applicable to anyone in society.

For this reason, the interview sub-study restricted participation to older South Asian and Caucasian women, aged 60-70 years old, dwelling within a 20 mile radius of Guildford. There would not have been enough subjects available to put further restrictions on entry to the study. For example, choosing just Muslims, although making for a ‘tighter’ analysis, would have made it difficult to recruit enough women to the study, and would have made the findings less applicable to non-Muslim South Asians. Because of language difficulties for most women in this older South Asian age group, especially in the lower Socio-economic classes, this meant that the South Asian participants tended to be from a medium high socio-economic class. Although this means they are not necessarily representative of much of the general South Asian population living in the UK it does allow them to be comparable to the Caucasian women in the study, who were also of a moderately high socio-economic status, thus removing social class as a confounding factor in the analysis.
CHAPTER 3 - Vitamin D status: within & between year change
Part I: Within year change in 25(OH)D and its effects on bone health
n.b. This part of Chapter 3 has been published:

See Appendix II- Publications and Conference abstracts for full paper

3.1 Introduction

It is known that the wavelength of the sun reaching the earth at its zenith varies seasonally, for geographical areas at latitudes far from the equator. This leads to variation in 25(OH)D concentration by season in people dwelling in these areas. Some individuals show more seasonal variation than others. More variation is seen in those with puler skin, or who expose themselves more to the sun than seen in those with darker skin, or who get lower sun exposure. South Asians (Finch et al. 1992; Macdonald et al. 2006; Darling et al. 2013) and older people from all ethnic groups (Lester et al. 1977; Lagunova et al. 2009) show less pronounced seasonal variation in their 25(OH)D concentration than other population sub-groups including younger adults and Caucasians. This seasonal change in 25(OH)D may be of concern for health. It has been hypothesised by Vieth (2004) that seasonal change in 25(OH)D, may have an adverse effect on the activity of the enzymes that control vitamin D metabolism (Vieth 2004). Thus, individuals with large seasonal change in 25(OH)D concentration may have sub-optimal 1,25(OH)_2D concentration for much of the year. There is no evidence to date as to whether regular large seasonal changes in 25(OH)D concentration have any effect on health. There has been some suggestion of potential harm, however, based on findings of increased risk of prostate and pancreatic cancers (Tuohimaa et al. 2004; Brock et al. 2010) and findings of increased mortality (Michaelsson et al. 2011) in individuals with high vitamin D status. It has been proposed that these detrimental effects could be due to seasonal changes in 25(OH)D rather than high 25(OH)D itself (Vieth 2004). This intriguing hypothesis proposed to explain the increased cancer risk (Vieth 2004) begs the question as to whether seasonal fluctuation or ‘cycling’ of 25(OH)D could also be detrimental to other aspects of health. It is unknown whether these large seasonal fluctuations in 25(OH)D may have an impact on bone health.

Establishing an effect of seasonal change in 25(OH)D on bone health will be clinically relevant in order to inform the advice given by clinicians and public health providers, specifically with regard to vitamin D supplementation practices. If large seasonal changes in 25(OH)D status increase bone resorption, then winter only supplementation of vitamin D may be warranted. This would move the
Vitamin D status

objective of vitamin D supplementation away from just raising 25(OH)D concentrations to also ‘blunting’ the seasonal change. Furthermore, clinical aims may be to achieve a 25(OH)D concentration which is both stable and of optimal concentration (Vieth 2009).

This study aimed to assess whether there is an association between bone resorption and the amount of seasonal change in 25(OH)D concentration. It was hypothesised that individuals showing a high degree of seasonal cycling of 25(OH)D would show increased bone resorption, as evidenced by both increased serum c-telopeptide [sCTX] and serum parathyroid hormone [sPTH] concentration.

3.2 Methods

3.2.1 Statistical methods

This analysis uses 2006 data from the original D-FINES study, from both premenopausal and postmenopausal women, to assess within year change in 25(OH)D. The premenopausal and postmenopausal demographic characteristics for the whole 2006 cohort can be seen in Appendix Y. Results are presented as mean (SD). Information about the recruitment of participants, biochemical measurements and study design for this study has been presented in chapter 2 (Section 2.1). Only extra information relevant the statistical aspects of this particular analysis will be discussed here.

3.2.2 Non-Linear Mixed Modelling Analysis

The original study design for the D-FINES data was to allow comparisons between vitamin D status between seasons and ethnic-menopausal groups, rather than to assess seasonal change in detail over the course of the year. For this subsequent analysis, where assessment of seasonal change was required in more detail, the actual visit date for biochemical measurements, rather than season, was used for each measurement and this data was pooled.

A non-linear mixed modelling approach was used to assess the hypothesis that individuals with a high degree of seasonal cycling of 25(OH)D would show increased bone resorption, as evidenced by increased serum c-telopeptide [sCTX] and serum parathyroid hormone [sPTH] concentration. The sPTH data and 25(OH)D were not normally distributed so were log transformed. Measurements for sPTH, sCTX and 25(OH)D were approximately equally spaced over a year with precise visit dates used in the analysis, rather than month or season. Demographic data were drawn from baseline data only. The four ethnic-menopausal subject groups in our dataset were postmenopausal Caucasian,
Vitamin D status of premenopausal Caucasian, postmenopausal South Asian and premenopausal South Asian and were entered into the model as 3 dummy variables, contrasting the first group (postmenopausal Caucasian) with the remainder. BMI was entered into the model as it is known to be associated with overall 25(OH)D (Greene-Finestone et al. 2011; Brock et al. 2010), and seasonal change in 25(OH)D (Lagunova et al. 2009). As potential confounders, at all times, BMI and ethnic-menopausal group were included in the model. It was important to control for ethnicity and menopausal status as these two factors are also known to be associated with differences in vitamin D status and vitamin D metabolism.

The modelling procedure was as follows: To investigate constants of proportionality for the first dependent variable (sPTH), the data were analysed for all the participants who had a complete set of 4 data points for log sPTH and log 25(OH)D, as well as baseline data for BMI and ethnic/menopausal group. This was a total of n =200 women (n=96, n=65, n=21 and n=18 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively). The procedure for the sCTX analysis was the same as for sPTH (see above). The equivalent data in this analysis was for n= 60 women (n=15, n=18, n=15 and n=12 respectively in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians).

The model was then used to assess whether sPTH concentrations were proportional to the amplitude of seasonal variation in log 25(OH)D divided by the mean log 25(OH)D. It was important to adjust the amplitude by the mean log 25(OH)D concentration (mesor), in order to control for the confounding effects of overall mean 25(OH)D concentration. The individual participant’s four data points for log 25(OH)D were modelled as a mean level specific to that participant, to which was added a sine wave of amplitude and angular off-set both also specific for that participant, as well as a random normally distributed error term. The two participant-specific variables, mean level and angular offset were modelled as mixed random effects with unstructured variance-covariance matrix.

The sPTH data were simultaneously regressed as sets of four within participant repeated measures (with unstructured variance covariance matrix, also encompassing the effects of the above mentioned two participant-specific variables) against the independent variables (ratio of amplitude to mean of log 25(OH)D (i.e. amplitude/mesor), average yearly level of 25(OH)D, ethnic/menopausal status category and BMI. The whole procedure was repeated for sCTX as the dependent variable. The non-linear mixed modelling analysis was conducted using the NL MIXED procedure, of the SAS (SAS Institute, Cary, NC, USA) software suite. Regression parameters significantly different from zero within the limits of the conventional 95% confidence interval were deemed statistically significant.
3.3 Results

3.3.1 Participant Characteristics

Table 3.1 shows the baseline anthropometry and biochemistry of the cohort (n=367) the participants were drawn from, including 25(OH)D, sPTH and sCTX concentration in each season.

Table 3.1. Characteristics of participants in D-FINES cohort (n=367)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>367</td>
<td>48.2</td>
<td>14.4</td>
<td>19.98</td>
<td>76.42</td>
</tr>
<tr>
<td>Body Mass Index (BMI) (kg/m²)</td>
<td>365</td>
<td>26.4</td>
<td>5.1</td>
<td>16.40</td>
<td>36.40</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>365</td>
<td>69.6</td>
<td>12.7</td>
<td>44.71</td>
<td>94.49</td>
</tr>
<tr>
<td>Height (m)</td>
<td>365</td>
<td>1.6</td>
<td>0.1</td>
<td>1.40</td>
<td>1.80</td>
</tr>
<tr>
<td>Dietary calcium (mg)±</td>
<td>286</td>
<td>833</td>
<td>308</td>
<td>229.32</td>
<td>1436.68</td>
</tr>
<tr>
<td>Summer 25(OH)D (nmol/L)</td>
<td>346</td>
<td>58.4</td>
<td>27.1</td>
<td>5.28</td>
<td>111.52</td>
</tr>
<tr>
<td>Autumn 25(OH)D (nmol/L)</td>
<td>281</td>
<td>51.1</td>
<td>24.7</td>
<td>2.69</td>
<td>99.51</td>
</tr>
<tr>
<td>Winter 25(OH)D (nmol/L)</td>
<td>253</td>
<td>38.4</td>
<td>18.0</td>
<td>3.12</td>
<td>73.68</td>
</tr>
<tr>
<td>Spring 25(OH)D nmol/L</td>
<td>248</td>
<td>42.7</td>
<td>22.0</td>
<td>-0.42</td>
<td>85.82</td>
</tr>
<tr>
<td>Summer sCTX ng/mL</td>
<td>65</td>
<td>0.34</td>
<td>0.16</td>
<td>0.03</td>
<td>0.65</td>
</tr>
<tr>
<td>Autumn sCTX ng/mL</td>
<td>65</td>
<td>0.34</td>
<td>0.15</td>
<td>0.05</td>
<td>0.63</td>
</tr>
<tr>
<td>Winter sCTX ng/mL</td>
<td>65</td>
<td>0.33</td>
<td>0.15</td>
<td>0.04</td>
<td>0.62</td>
</tr>
<tr>
<td>Spring sCTX ng/mL</td>
<td>65</td>
<td>0.35</td>
<td>0.16</td>
<td>0.04</td>
<td>0.66</td>
</tr>
<tr>
<td>Summer sPTH pmol/L</td>
<td>345</td>
<td>2.8</td>
<td>2.0</td>
<td>3.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Autumn sPTH pmol/L</td>
<td>291</td>
<td>2.8</td>
<td>2.0</td>
<td>3.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Winter sPTH pmol/L</td>
<td>244</td>
<td>3.0</td>
<td>2.1</td>
<td>3.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Spring sPTH pmol/L</td>
<td>258</td>
<td>2.8</td>
<td>2.0</td>
<td>3.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

sPTH=serum parathyroid hormone; sCTX=serum C-telopeptide of collagen; 25(OH)D=serum 25-hydroxyvitamin D; summer to winter 25(OH)D ratio=winter 25(OH)D-summer 25(OH)D; n=number of participants with measurement. ≠ n=144, n=135, n=42 and n=46 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively.
Vitamin D status

The women had a mean BMI of 26.4 Kg/m² (5.1), thus were classified as overweight. They also had a mean age of 48.2 (14.4) years and a dietary calcium intake of 833(308) mg/d. 25(OH)D concentration ranged from 58.4-38.4 nmol/L, depending on season. Concurrently, the ranges for sPTH and sCTX concentrations by season were 3.1-2.9 pmol/L and 0.3-0.6 ng/mL respectively. Tables 3.2 and 3.3 show the same information, but for the subsets of the cohort who were included in the sPTH and sCTX analyses due to having complete data for all relevant variables (n=200, sPTH; n=60, sCTX). As can be seen from comparing Table 3.1 (entire cohort) with that of Table 3.2 (sPTH analysis) and Table 3.3 (sCTX analysis), the women included in the sPTH and sCTX analyses were representative of the entire cohort. They had similar age, BMI and dietary calcium intake to that of the original cohort.

Table 3.2: Characteristics of participants- (n=200) in the log sPTH analysis ≠

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>200</td>
<td>50.6</td>
<td>12.9</td>
<td>25.32</td>
<td>75.88</td>
</tr>
<tr>
<td>Body mass index (BMI) (Kg/m²)</td>
<td>200</td>
<td>26.2</td>
<td>4.7</td>
<td>16.99</td>
<td>35.41</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>200</td>
<td>68.8</td>
<td>12.0</td>
<td>45.28</td>
<td>92.32</td>
</tr>
<tr>
<td>Height (m)</td>
<td>200</td>
<td>1.62</td>
<td>0.06</td>
<td>1.50</td>
<td>1.74</td>
</tr>
<tr>
<td>Dietary calcium (mg)</td>
<td>186</td>
<td>862</td>
<td>329</td>
<td>217.16</td>
<td>1506.84</td>
</tr>
<tr>
<td>Summer 25(OH)D (nmol/L)</td>
<td>200</td>
<td>59.2</td>
<td>27.7</td>
<td>4.91</td>
<td>113.49</td>
</tr>
<tr>
<td>Autumn 25(OH)D (nmol/L)</td>
<td>200</td>
<td>50.7</td>
<td>24.3</td>
<td>3.07</td>
<td>98.33</td>
</tr>
<tr>
<td>Winter 25(OH)D nmol/L</td>
<td>200</td>
<td>38.1</td>
<td>17.5</td>
<td>3.80</td>
<td>72.40</td>
</tr>
<tr>
<td>Spring 25(OH)D nmol/L</td>
<td>200</td>
<td>43.1</td>
<td>22.5</td>
<td>-1.00</td>
<td>87.20</td>
</tr>
<tr>
<td>Summer sCTX ng/mL</td>
<td>59</td>
<td>0.34</td>
<td>0.16</td>
<td>0.03</td>
<td>0.65</td>
</tr>
<tr>
<td>Autumn sCTX ng/mL</td>
<td>59</td>
<td>0.34</td>
<td>0.16</td>
<td>0.03</td>
<td>0.65</td>
</tr>
<tr>
<td>Winter sCTX ng/mL</td>
<td>59</td>
<td>0.33</td>
<td>0.16</td>
<td>0.02</td>
<td>0.64</td>
</tr>
<tr>
<td>Spring sCTX ng/mL</td>
<td>59</td>
<td>0.36</td>
<td>0.17</td>
<td>0.03</td>
<td>0.69</td>
</tr>
<tr>
<td>Summer sPTH pmol/L</td>
<td>200</td>
<td>2.90</td>
<td>2.00</td>
<td>3.70</td>
<td>1.7</td>
</tr>
<tr>
<td>Autumn sPTH pmol/L</td>
<td>200</td>
<td>2.80</td>
<td>2.00</td>
<td>3.70</td>
<td>1.7</td>
</tr>
<tr>
<td>Winter sPTH pmol/L</td>
<td>200</td>
<td>3.00</td>
<td>2.10</td>
<td>3.80</td>
<td>1.7</td>
</tr>
<tr>
<td>Spring sPTH pmol/L</td>
<td>200</td>
<td>2.80</td>
<td>2.00</td>
<td>3.60</td>
<td>1.6</td>
</tr>
</tbody>
</table>

sPTH=serum parathyroid hormone; sCTX=serum C-telopeptide of collagen; 25(OH)D=serum 25-hydroxyvitamin D; summer to winter 25(OH)D ratio=winter 25(OH)D-summer 25(OH)D, n=number of participants with measurements. ≠ n=96, n=65, n=21 and n=18 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively.
Vitamin D status

Also, for the sPTH analysis, 25(OH)D and sPTH concentrations were similar to that of the whole cohort. However, for the sCTX analysis, 25(OH)D was slightly lower and sPTH slightly higher than in the original cohort. This was due to a more even split of South Asian and Caucasian women in the sCTX analysis. This is in contrast to the sPTH analysis whereby there were a higher number of Caucasians than South Asians.

Table 3.3: Characteristics of participants- (n=60) in the sCTX analysis

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60</td>
<td>47.7</td>
<td>12.4</td>
<td>23.40</td>
<td>72.00</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m²)</td>
<td>60</td>
<td>26.0</td>
<td>4.1</td>
<td>17.96</td>
<td>34.04</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60</td>
<td>66.5</td>
<td>10.1</td>
<td>46.70</td>
<td>86.30</td>
</tr>
<tr>
<td>Height (m)</td>
<td>60</td>
<td>1.60</td>
<td>0.06</td>
<td>1.48</td>
<td>1.72</td>
</tr>
<tr>
<td>Dietary calcium (mg)±</td>
<td>52</td>
<td>857</td>
<td>417</td>
<td>39.68</td>
<td>1674.3</td>
</tr>
<tr>
<td>Summer 25(OH)D (nmol/L)</td>
<td>60</td>
<td>47.8</td>
<td>25.3</td>
<td>-1.79</td>
<td>97.39</td>
</tr>
<tr>
<td>Autumn 25(OH)D nmol/L</td>
<td>60</td>
<td>41.2</td>
<td>25.3</td>
<td>-8.39</td>
<td>90.79</td>
</tr>
<tr>
<td>Winter 25(OH)D nmol/L</td>
<td>60</td>
<td>33.9</td>
<td>20.4</td>
<td>-6.08</td>
<td>73.88</td>
</tr>
<tr>
<td>Spring 25(OH)D nmol/L</td>
<td>60</td>
<td>36.9</td>
<td>20.9</td>
<td>-4.06</td>
<td>77.86</td>
</tr>
<tr>
<td>Summer sCTX ng/mL</td>
<td>60</td>
<td>0.34</td>
<td>0.16</td>
<td>0.03</td>
<td>0.65</td>
</tr>
<tr>
<td>Autumn sCTX ng/mL</td>
<td>60</td>
<td>0.34</td>
<td>0.16</td>
<td>0.03</td>
<td>0.65</td>
</tr>
<tr>
<td>Winter sCTX ng/mL</td>
<td>60</td>
<td>0.33</td>
<td>0.16</td>
<td>0.02</td>
<td>0.64</td>
</tr>
<tr>
<td>Spring sCTX ng/mL</td>
<td>60</td>
<td>0.35</td>
<td>0.17</td>
<td>0.02</td>
<td>0.68</td>
</tr>
</tbody>
</table>

sPTH=serum parathyroid hormone; sCTX=serum C-telopeptide of collagen; 25(OH)D=serum 25-hydroxyvitamin D; summer to winter 25(OH)D ratio=winter 25(OH)D-summer 25(OH)D; n=number of participants with measurements.

≠n=15, n=18, n=15 and n=12 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively.
3.3.2 Within year change in 25(OH)D- Descriptives

Monthly data for 25(OH)D by ethnic group can be seen in Figure 3.1. Between February (nadir) and September (peak) the Caucasians increased in 25(OH)D concentration from 41.9nmol/L to 66.4 nmol/L (+158%). The equivalent data for Asians was an increase from 20.7nmol/L to 31.3nmol/L (+151%). The percentage of change in 25(OH)D was similar in the two ethnic groups, but with a large actual change in 25(OH)D concentration seen in the Caucasians, due to a higher starting level\(^3\).

Figure 3.1: Monthly data for the 2006-2007 study by ethnic group

3.3.3 Non-Linear Mixed Modelling

The regression analysis is summarised in Table 3.4, including the effect sizes for the main model parameters, here defined as the absolute value of the quotient of the estimated value and the standard error. Thus defined, the effect size for a parameter is only an indication of how significantly different from 0 the value of the parameter is, (i.e. it is an indication of how necessary it is to include, as opposed to excluding, that parameter in the model). However, apart from identifying the importance of including the parameter in the model, the effect size conveys no other information about the functioning of the model.

For log sPTH, the regression coefficient and SE for the amplitude/mesor ratio of 25(OH)D showed a significant positive relationship, after adjustment for confounders (BMI and ethnic/menopausal group), and indicates that the amplitude/mesor parameter for 25(OH)D, as well as overall 25(OH)D concentration were significant predictors of log sPTH concentration.

\(^3\) It was not possible to conduct repeated measures inferential statistics (e.g. ANOVA) on this data due to no individuals having more than 4 data points out of 12, due to data collected once per season.
Table 3.4: Regression model for log sPTH (n=200) and sCTX (n=60)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>sPTH n=200(^a)</th>
<th>sCTX n=60(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicator (0 1) variable for (PRE C) v (POST C)</td>
<td>-0.092</td>
<td>0.511</td>
</tr>
<tr>
<td>Indicator (0 1) variable for (POST A) v (POST C)</td>
<td>0.511</td>
<td>0.052</td>
</tr>
<tr>
<td>BMI (Body mass index) kg/m(^2)</td>
<td>0.037</td>
<td>0.057</td>
</tr>
<tr>
<td>25(OH)D regression coefficient</td>
<td>-0.018</td>
<td>0.057</td>
</tr>
<tr>
<td>25(OH)D ratio (amplitude/mesor)</td>
<td>0.057</td>
<td>0.057</td>
</tr>
<tr>
<td>-2 log likelihood</td>
<td>1330.7</td>
<td>292.0</td>
</tr>
</tbody>
</table>

\(^a\) n=96, \(^b\) n=65, \(^c\) n=21 and \(^d\) n=18 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively. ** Body Mass Index. ± n=15, n=18, n=15 and n=12 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively.
Effect sizes were similar for both the amplitude/mesor ratio and the overall 25(OH)D concentration. Similarly, for sCTX, the regression coefficient for amplitude/mesor ratio was also statistically significant, as was that for overall 25(OH)D concentration. As with sPTH, the effect sizes were similar for overall 25(OH)D concentration and the amplitude/mesor ratio.

For log sPTH, the regression coefficient (and SE) for the amplitude/mesor ratio of 25(OH)D were 0.057 (0.003) with a 95% confidence interval (0.051, 0.063); \( p<0.0001 \). The effect size was 19.0, which means that the estimated value for that parameter was 19 standard errors of the estimate removed from 0. This shows a significant positive relationship, after adjustment for confounders (BMI and ethnic/menopausal group), and indicates that the amplitude/mesor parameter for 25(OH)D was a significant predictor of log sPTH concentration. For sPTH the regression coefficient (SE) for the level of 25(OH)D was -0.018 (0.001) with a 95% confidence interval of (-0.020, -0.016); \( p<0.0001 \). The effect size was 18.0, marginally smaller than for the coefficient referred to immediately above.

For sCTX, the regression coefficient for amplitude/mesor ratio of 25(OH)D had an estimated value of 0.528 (95% confidence interval 0.418, 0.638; \( P\leq0.0001 \)) which was also statistically significant so that conclusions analogous to the above follow. The effect size was 9.3, which means that the estimated value for that parameter is 9.3 standard errors of the estimate removed from 0. For sCTX the regression coefficient (SE) for the level of 25(OH)D was -0.105 (0.014) with a 95% confidence interval of (-0.132, -0.078); \( p<0.0001 \). The effect size was 7.5, marginally smaller than that for the coefficient referred to immediately above. A post-hoc power calculation showed that the study results showed adequate power in excess of 99.9% for investigating the relationship for both dependent variables (sCTX and sPTH) and the seasonal variation in serum 25(OH)D, adjusting for confounding effects.

### 3.4 Discussion

#### 3.4.1 Stability of 25(OH)D concentration throughout the year in Asians

This study shows that Asians have lower 25(OH)D concentrations than Caucasians throughout the year, however the 25(OH)D levels are also slightly more stable. This supports previous work showing a blunted seasonal variation in 25(OH)D in South Asians (Finch et al. 1992; Darling et al. 2013; Macdonald et al. 2011). The slightly more stable 25(OH)D in Asians can be explained by their lower UVB exposure (Darling et al, 2013), as compared with Caucasians. Lower sun exposure means 25(OH)D concentrations are less responsive to seasonal change. This also explains why
25(OH)D is lower on average in Asians, as to a certain degree they are missing the largest source of vitamin D. However, this stability may have advantages to bone health, as will be discussed next.

### 3.4.2 Effects of within year change in 25(OH)D on bone resorption

This is the first study, to the authors’ knowledge, that has examined the association between seasonal change in 25(OH)D and a marker of bone resorption. A significant positive relationship was observed between the seasonal change in 25(OH)D and sPTH, supporting our original hypothesis suggesting that those individuals with a higher seasonal change in 25(OH)D had a higher sPTH. There was also a statistically significant association between seasonal change in 25(OH)D and bone resorption, as measured by sCTX, so that similar conclusions to the above are applicable.

The above findings suggest that the higher sPTH seen with increased seasonal change in 25(OH)D may translate into alterations in bone resorption. The result for sCTX are not surprising. A concomitant increase in sCTX would be predicted due to the increased bone resorption implicated by increased sPTH levels. The trends observed for sPTH and sCTX in the current study lend support to Vieth’s hypothesis (Vieth 2004) that large seasonal changes in 25(OH)D might be associated with some adverse health outcomes. In this study, for both sPTH and sCTX, seasonal fluctuation (as expressed by the amplitude/mesor ratio) had an (albeit marginally) larger predictive ability in explaining sPTH and sCTX than did the average concentration of 25(OH)D (as assessed by respective coefficient effect sizes). Thus, in this dataset, seasonal variation in 25(OH)D status had a marginally statistically more significant impact on sPTH and sCTX concentration than did overall 25(OH)D concentration.

It is important to know if seasonal cycling of 25(OH)D is detrimental to health, in order to inform supplementation advice for vitamin D. Specifically, it raises the question of whether year round supplementation of vitamin D, or winter only supplementation should be recommended. The clinical and public health implications of this study might suggest that wintertime only supplementation may be beneficial in order to blunt the rhythm of 25(OH)D, keeping 25(OH)D levels consistent throughout the year. In addition, it may be essential to understand seasonal variation in 25(OH)D to assist in the interpretation of some of the adverse effects reported in the literature in regard to high serum concentrations of 25(OH)D. Specifically, it may be crucial to separate the effects of high levels of 25(OH)D per se from those of seasonal variation in order to establish guidelines for optimal 25(OH)D concentrations, which remain a topic of on-going debate in the vitamin D field. Findings from the current study suggest that seasonal variation, as well as the overall concentration, of 25(OH)D may need to be considered when assessing optimal vitamin D status. However, before these findings can be translated clinically, and changes to advice for vitamin D supplementation
made, further investigation is required as to whether the increased bone resorption seen here is also accompanied by increased bone formation. This is because an assessment is required as to whether the results are indicative of an overall increased bone turnover, which is not necessarily detrimental to bone health. Indeed, small perturbations in sCTX and sPTH may not be harmful to bone health in the long term. These results may only be of clinical significance if seasonal cycloing can be found in subsequent research to lead to reduced BMD, and detrimental effects on skeletal architecture, leading to increased fracture risk. It is unknown from this data alone whether this would be the case.

3.4.3 Limitations

A primary limitation of this study is that for the bone resorption assessments (sCTX) only a subset of the participants were utilised and that the study was not originally powered to assess seasonal change and bone resorption. Another limitation is that the study findings are generalisable only to Caucasian and South Asian women, but may not be applicable to other ethnic groups due to potential differences in vitamin D metabolism that may affect seasonal changes in 25(OH)D, sPTH and sCTX. A larger sample size for bone markers will be more informative to clarify the relationship between seasonal fluctuation in 25(OH)D and bone resorption. In future work, it will be important to assess markers of bone formation as well as resorption as overall bone turnover is important for bone health, not just bone resorption. It would also be useful in longitudinal research studies to assess whether structural changes in bone are associated with seasonal changes in 25(OH)D, in order to determine possible chronic effects on bone health, such as lowered BMD and increased fracture risk. Even if seasonal fluctuation in 25(OH)D is detrimental to the activity of the bone vitamin D hydroxylase enzymes, there could still be physiological adaptation to this in the long term.

3.5 Conclusion

Asians have lower, but more stable 25(OH)D concentrations throughout the year. This analysis shows that greater seasonal cycling of 25(OH)D is associated with increased sPTH concentration and with bone resorption. In terms of public health, this finding suggests vitamin D supplements should not necessarily be taken all year round and there may be justification for ‘blunting’ the rhythm of 25(OH)D concentration over the course of the year. If these findings can be replicated in a larger sample then they may suggest that a strategy of wintertime only supplementation is required. Furthermore, it suggests seasonal variation in 25(OH)D, as well as overall concentration, should be considered when making recommendations as to optimal concentrations of 25(OH)D for health.
Part II: What are the predictors of between yearly change in 25(OH)D?
Vitamin D status

3.6 Introduction

It is known that the main contributor to 25(OH)D concentration is UVB exposure from sunlight. Most of the body’s vitamin D is made from sunlight in the skin and is converted to 25(OH)D in the liver by hydroxylation. Dietary vitamin D is known to be the second main source, whereby vitamin D$_2$ and D$_3$ enter the bloodstream via the gut, and are also hydroxylated in the liver to form 25(OH)D. However, it is not clear what the relative contributions of these factors are in explaining change in 25(OH)D status in populations and individuals between years. Also, it is relatively unknown as to what degree the importance of specific sub classes of these factors are, (e.g. holiday time abroad, as a subset of sun exposure in general). It is also unknown how much populations and individuals change in 25(OH)D over a period of 5-10 years. Recent work suggests either a small decline in 25(OH)D over a time period of years, in some sub-population groups (Ginde, Liu, and Camargo 2009) or that there is no change over a period of years (Jorde et al. 2010). The discrepancies between these studies are likely to be due to differences in the population being studied and the length of follow up. There is no known longitudinal study that has measured both change in 25(OH)D and all the main parameters that affect vitamin D status (e.g. sun exposure, dietary intake, holiday time, dress style etc). Also, there have been very few longitudinal studies of vitamin D status that cover a period of years in the UK, with no surveys focused on UK ethnic minority groups. Moreover, some ethnic groups, such as South Asians are even underrepresented in the main UK surveys of 25(OH)D status (e.g. NDNS). It is important to understand the factors influencing 25(OH)D status in the UK population in order to tailor interventions to improve vitamin D status. Using data collected in 2006 and 2010 (both the D-FINES study and its follow up measurement) this analysis aimed to describe the changes in vitamin D status in the postmenopausal D-FINES women and to elucidate the main contributing factors explaining any change in 25(OH)D status.

3.7 Methods

3.7.1 Study design, recruitment and procedures

The study design was a longitudinal comparison between the data collected during D-FINES 2006 and the follow up measurement collected in 2010. Only women not on vitamin D supplements at either time point were included in this analysis. Comparison data for all the women, including those on supplements, can be found in Appendix Z. Also, the 2010 immunoassay 25(OH)D data (rather than HPLC data) was used for this analysis, to make a valid comparison with the 2006 data.
Details of study design, participant recruitment and procedures for the 2006 and 2010 analysis are as previously presented in chapter 2 (Sections 2.1. and 2.2.). This analysis uses just the postmenopausal cohort (both South Asian and Caucasian) from 2006 and 2010.

### 3.7.2 Biochemical, UVB and dietary measurements

To enable a valid comparison between the two time points, the same brand and type of 25(OH)D immunoassay (IDS immunodiagnostics) were used in 2010 as was used in 2006. Sensitivity and specificity values for the IDS immunoassay were presented previously in chapter 2 (Section 2.1.4). The same dosimeter badges, food diaries and study protocols were used in the two years, as well as the same questionnaires and equipment to assess lifestyle and anthropometry.

### 3.7.3 Statistical analysis

Mean daily vitamin D and calcium intakes were calculated by summing the total intakes of these nutrients as reported in the food diary, then dividing by the number of days the diary covered. For vitamin D intake, 3 outliers were noted (D091 (5.2µg/d), D080 (7.5µg/d), D252 (9.6µg/d). These comprised the top 5% of values and were also deemed unfeasible intakes of vitamin D. Therefore, they were removed from the analysis. Vitamin D and calcium intakes from 2006 were not normally distributed so were log transformed prior to analysis. Energy adjusted daily values for dietary vitamin D and calcium were also calculated by dividing the value for these nutrients by that for energy intake (kJ). Thus, these nutrients were expressed per kJ of dietary energy, per day. Energy adjusted data for vitamin D intake in 2006 was not normally distributed, so was log transformed prior to analysis. For the energy adjusted analysis, previous outliers for the vitamin D intake data were retained in the analysis, as energy adjustment may have normalised these values.

Standard Erythemal Dose (SED) was calculated by the formula given previously in chapter 2 (Section 2.1.5). Total weekly UVB exposure (in SED) was calculated by adding the SED value for the badge for weekend days (2 days) to that of the badge for weekdays (5 days). To calculate mean daily UVB exposure (in SED), the total weekly UVB exposure was divided by 7. Some values had to be removed from the analysis, specifically all values from participants D102 and D201. This was because the badges had become damaged during participant use, and it was unclear whether the badges had been worn on weekdays or the weekend.

Total holiday days were calculated from participant self-reports of the number of days spent on holiday (inside and outside the UK) (see appendix J for questionnaire items regarding holidays). Total vitamin D producing holiday days (2006) were not normally distributed so were log
transformed prior to analysis. In addition, a secondary holiday score was calculated based on the likelihood of producing vitamin D at the specified time and location. For example, a holiday to Poland in August would be counted as ‘vitamin D producing’ but not a holiday to Germany in February. This score consisted of a count of the number of holiday days that were deemed to be ‘vitamin D producing’.

For the regression analysis, change parameters for continuous variables (i.e. 25(OH)D concentration, BMI, dietary vitamin D, dietary calcium, UVB exposure, number of holiday days) were calculated by subtracting the 2006 value from the 2010 value. The exception was age, whereby yearly change was a constant (change in age = 4) for all participants. Hence, actual participant age in 2010 was entered into the model. Ethnicity was entered as a dummy variable (0=Caucasian, 1=Asian). Parameters were added into the model together, rather than by a stepwise method. This was due to the known problem of stepwise methods relying solely on statistical correlation when allowing variables into the model, omitting considerations of variables’ biological relevance. It was considered that there were not enough variables to deem a hierarchical regression model valid, thus variables were all entered at the same time into the analysis. PTH data (2010) and 25(OH)D data (2006) were log transformed prior to analysis to normalise the distribution. SPSS v19 (Chicago, IL) was used for all the statistical analyses in the remainder of this chapter, with Graph Pad Prism v 6.01 (San Diego, CA) being used for producing the figures. Win Diets Research (Robert Gordon University, Aberdeen, UK) was used to analyse the food diary data.

3.8 Results

3.8.1 Participant characteristics

Participant characteristics for the postmenopausal cohort are presented in Appendix Y (2006), and Appendix AA (2010) and will not be repeated here. However, the details of the specific women included in the two regression models are listed here (Tables 3.5 and 3.6), as these samples are likely to deviate from the characteristics of the whole cohort, due to the requirement of having complete data for a relatively large number of variables.
Table 3.5: Participant Characteristics- Postmenopausal women in regression analyses- model 1  
(n=36)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasian</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  SD</td>
<td>N  Mean  SD</td>
</tr>
<tr>
<td>Age</td>
<td>30 65.1  4.2</td>
<td>6 63.3  3.5</td>
</tr>
<tr>
<td>IMD</td>
<td>30 16.3  10.6</td>
<td>6 9.8  13.9</td>
</tr>
<tr>
<td>Dietary Vitamin D (μg/d)</td>
<td>30 2.9  1.6</td>
<td>4 2.3  1.3</td>
</tr>
<tr>
<td>Dietary Calcium (mg/d)</td>
<td>30 802.4  265.9</td>
<td>4 742.0  74.2</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>30 61.8  18.5</td>
<td>6 36.0  11.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30 26.1  4.6</td>
<td>6 27.7  4.1</td>
</tr>
<tr>
<td>Mean daily UVB exposure (SED)</td>
<td>24 0.39  0.1</td>
<td>6 0.40  0.1</td>
</tr>
<tr>
<td>Vitamin D producing holiday days</td>
<td>13 25.4  30.1</td>
<td>4 39.5  60.4</td>
</tr>
<tr>
<td>Walking (mins/d)≠</td>
<td>22 70.5  49.3</td>
<td>6 53.3  62.2</td>
</tr>
</tbody>
</table>

*=independent samples t-test ≠ Index of physical activity

Table 3.6: Participant Characteristics 2010- Postmenopausal women in regression analyses- model 2  
(n=22)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasian</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  SD</td>
<td>N  Mean  SD</td>
</tr>
<tr>
<td>Age</td>
<td>22 65.3  4.0</td>
<td>2 63.0  2.8</td>
</tr>
<tr>
<td>IMD</td>
<td>22 16.5  11.0</td>
<td>2 3.9  2.5</td>
</tr>
<tr>
<td>Dietary Vitamin D (μg/d)</td>
<td>22 2.6  1.4</td>
<td>2 3.1  0.5</td>
</tr>
<tr>
<td>Dietary Calcium (mg/d)</td>
<td>22 774.0  292.2</td>
<td>2 705.0  70.7</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>22 63.0  20.3</td>
<td>2 36.8  14.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22 26.4  5.2</td>
<td>2 31.1  4.8</td>
</tr>
<tr>
<td>Mean daily UVB exposure (SED)</td>
<td>22 0.37  0.1</td>
<td>2 0.43  0.1</td>
</tr>
<tr>
<td>Vitamin D producing holiday days</td>
<td>7 26.7  17.3</td>
<td>2 10.5  5.0</td>
</tr>
<tr>
<td>Walking (mins/d)≠</td>
<td>14 63.6  47.9</td>
<td>2 30.0  0.0</td>
</tr>
</tbody>
</table>

*=independent samples t-test ≠ Index of physical activity; ±not able to run Independent t-test due to n=2 in Asian group

There were no significant ethnic differences for any variable for participants included in regression model 1 (Table 3.5), except for 25(OH)D (P=0.002), which was higher in the Caucasians. It was not possible to assess ethnic differences in model 2 (Table 3.6) due to too low subject numbers in the Asian group. Of interest, comparing this data with that of the whole 2010 postmenopausal cohort (see Appendix AA) the Asians in the regression model show a trend for lower IMD scores (i.e. higher socio-economic status) and a lower BMI than the Asians in the whole 2010 cohort. Indeed, the Asians in the model 1 regression analysis had a mean (SD, n) for IMD of 9.76 (13.9, n=6), compared with 12.54 (12.3, n=19) for all Asians in the whole cohort. Concurrently, the Asian
women who had data in model 1 also had lower BMI, with a mean (SD, n): 27.7 (4.1, n=6) vs. 29.3 (4.5, n=21) in the whole cohort⁴.

### 3.8.2 Descriptives: 25(OH)D in summer 2006 and 2010

Independent t-tests showed that Asians had a lower mean 25(OH)D than Caucasians in 2006 (p<0.001) and 2010 (P=0.002) (Figure 3.2). There was little alteration in mean 25(OH)D when only women who attended both 2006 and 2010 were included in the analysis (Figure 3.3). Asians still had a lower mean 25(OH)D in 2006 (p<0.001) and 2010 (P=0.002).

![Figure 3.2: 25(OH)D in summer 2006 in postmenopausal women by ethnic group](image1)

![Figure 3.3: 25(OH)D in summer 2006 in postmenopausal women by ethnic group- paired data](image2)

As can be seen in Table 3.7, in 2006, more Asians than Caucasians were classified as severely deficient (<25nmol/L), (51% Asian vs. 0% Caucasian). However in 2010, the 25(OH)D status of Asians was higher than in 2006, with no women in either ethnic group being severely deficient. In both 2006 and 2010, 70% more Asians than Caucasians were classified as insufficient (<50nmol/L). Accordingly, in both years, no Asians were optimal (>75nmol/L), compared with one quarter to one third of Caucasians. In both years, the majority of the Caucasians, and all of the Asians fell under

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⁴ It was not possible to run inferential statistics for comparisons between these subsets and the whole cohort as the data were not independent (i.e. some women appeared in more than one table).
Vitamin D status

75nmol/L, thus being considered ‘sub optimal’ for 25(OH)D. The main trends here are for an improvement in 25(OH)D status in the Asian group, with more being classified as insufficient, rather than severely deficient in 2010 than 2006. In contrast, the percentage of Caucasian women under the different cut-off thresholds varied very little between the two years.

Table 3.7: % of postmenopausal women under 25(OH)D thresholds in 2006 and 2010

<table>
<thead>
<tr>
<th>25(OH)D nmol/L</th>
<th>&lt;25 Severe</th>
<th>&lt;50</th>
<th>&lt;75</th>
<th>Sub</th>
<th>&gt;75</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006 Caucasian (%) n=138</td>
<td>0</td>
<td>25</td>
<td>75</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Asian (%) n=38</td>
<td>51</td>
<td>95</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2010 Caucasian (%) n=33</td>
<td>0</td>
<td>24</td>
<td>68</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Asian (%) n=6</td>
<td>0</td>
<td>83</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

In terms of actual change in 25(OH)D from 2006-2010, Independent t-tests showed that Caucasian 25(OH)D concentrations fell by a mean (SD) of 1.5 (14.2) nmol/L, whereas Asians gained in 25(OH)D by 6.4 (7.7) nmol/L. However, there was no significant ethnic difference in the amount of change in 25(OH)D from 2006-2010 (t=1.305, P=0.201; Figure 3.4).

Figure 3.4: Change in serum 25(OH)D from 2006 to 2010 by ethnicity in post-menopausal women

Looking at quartiles of vitamin D status for each participant (as compared with the other subjects in their ethnic group), there was also no significant within subject differences for the quartile of vitamin D status they were in between 2006 and 2010 in Caucasians (Wilcoxon signed ranks test: Z=0.00; P>0.999; n=30) or in Asians (Wilcoxon signed ranks test: Z=-1.00; P=0.317, n=6).

3.8.2 Descriptives: PTH in summer 2006 and 2010

Independent t-tests showed that Asians had a significantly higher PTH than Caucasians in 2006 (t=2.985, P=0.003) but not 2010 (t=-0.902, P=0.370) (Figure 3.5).
Figure 3.5: PTH in summer 2006 in postmenopausal women by ethnic group

Using only data for women who attended both in 2006 and 2010, paired t-tests showed a significant reduction in PTH between 2006 and 2010 for Caucasians (t=5.244, P<0.001, n=53) and in Asians (t=2.784, P=0.014, n=16).

3.8.4 Mean daily UVB- summer 2006 vs. 2010

For between group effects, an independent t-test showed that there was a significantly higher mean daily UVB exposure in Caucasians than in Asians in 2006 (p<0.001; Figure 3.6). However, there was no ethnic difference in 2010 (P=0.837). In terms of within group effects, paired t-tests showed a significant difference in mean daily UVB exposure between 2006 and 2010 in Caucasians, with UVB being higher in 2006 (p<0.001). There was a reduction in mean daily UVB exposure by 0.88SED in Caucasians from 2006 to 2010. However, there was no significant difference in mean daily UVB exposure between the two years in Asians, despite a non-statistically significant increase in UVB exposure by 0.09SED (33%) in 2010 as compared with 2006 (P=0.503).

Figure 3.6: Mean daily UV exposure (SED) in 2006 and 2010 by ethnicity in post-menopausal women
3.8.5 Holiday days- summer 2006 vs. 2010

3.8.5.1 Total vitamin D producing holidays

Independent t-tests showed that there was a significant ethnic difference in total vitamin D producing holiday days in 2006, with Asians having a higher number of holiday days than Caucasians ($t=-3.208$, $P=0.006$). Caucasians had a mean (SD) of 20.4 (10.2) days, with Asians having a mean(SD) of 42.3 (13.6) days (Figure 3.7). However, there was no significant difference in vitamin D producing holiday days by ethnicity in 2010, ($t=-0.657$, $P=0.625$) with Caucasians having a mean (SD) of 27.6 (33.4) days, and the Asians having a mean of 68.5 (87.0) days.

![Figure 3.7: Mean total vitamin D producing holiday days in 2006 and 2010 for postmenopausal women, by ethnicity](image)

When including only women who had data in both 2006 and 2010, paired t-tests (within ethnic group) showed no significant change in total vitamin D producing holiday days between 2006 and 2010 in Caucasians ($t=-0.622$, $P=0.548$, n=11) or in Asians ($t=-3.040$, $P=0.202$, n=2). Caucasians changed from a mean (SD) of 1.25 (0.44) days to 1.36 (0.20) days. Asians changed from a mean (SD) of 0.85 (0.0) days to 1.74 (0.42) days.

3.8.5.2 Total holiday days

For 2006 total holiday days, an independent t-test showed that Asians had a significantly higher number of holiday days than Caucasians (26.33 (19.6) for Caucasians; 59.75 (34.7) Asians, ($P=0.011$), but in 2010 there was no significant ethnic difference ($P=0.497$) (Figure 3.8).
3.8.6 Vitamin D and calcium intakes

3.8.6.1 Actual vitamin D and calcium intakes in 2006 and 2010

Independent t-tests showed that Caucasians consumed significantly more vitamin D than Asians in 2006 ($t=3.313$, $P=0.001$; mean (SD): 3.1 (2.7) µg/d Caucasians vs. 1.89 (1.7) µg/d Asians). However, in 2010 there was no significant difference in vitamin D intake by ethnic group ($t=0.469$, $P=0.642$; mean (SD): 2.6 (1.3) µg/d Caucasians vs. 2.3 (1.3) µg/d Asians) (Figure 3.9). Also, for calcium intake, there was no ethnic difference for dietary calcium in 2006 ($t=1.170$, $P=0.224$) or in 2010 ($t=0.376$, $P=0.709$) (Figure 3.10).

Figure 3.8: Mean total holiday days in 2006 and 2010 for postmenopausal women, by ethnicity

When using only participants who had data for both 2006 and 2010, paired t-tests (within ethnic group) showed a significant difference between the total number of holiday days in 2006 and 2010 for Asians (mean (SD) difference = -32.5 (17.9), $n=4$, $P=0.036$) but not Caucasians (mean (SD) difference = -1.53 (32.7), $n=19$, $P=0.841$).
Figure 3.10: Mean calcium intake (µg/d) in 2006 and 2010 by ethnicity - unpaired data

However, when only women who attended in both 2006 and 2010 were included in the analysis, paired t-tests showed no significant difference in vitamin D intakes between the two ethnic groups in 2006 (t=0.737 P=0.467, n=30C and n=3A), or in 2010 (t=0.761, P=0.453, n=30C and n=3A)(Figure 3.11). Concurrently, there was still no ethnic difference between groups for calcium intake in 2006 (t=0.007 P=0.994, n=30C and n=3A) or 2010 (t=0.584 P=0.563, n=30C and n=3A).

Figure 3.11: Mean vitamin D intake (µg/d) in 2006 and 2010 by ethnicity - paired data

3.8.6.2 Energy adjusted vitamin D and calcium intakes in 2006 and 2010

Mean (SD, n) values for energy adjusted vitamin D and calcium intakes for each ethnic group are illustrated in Table 3.8. For energy adjusted vitamin D intake, there was no significant ethnic difference in 2006 (P=0.298) or 2010 (P=0.412). For energy adjusted calcium intake, there was no significant ethnic difference in 2006 (P=0.991) or in 2010 (P=0.581).
Table 3.8: Energy adjusted dietary vitamin D and calcium in 2006 and 2010

<table>
<thead>
<tr>
<th></th>
<th>Caucasians</th>
<th></th>
<th></th>
<th>Asians</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Energy adjusted vitamin D 2006*</td>
<td>35</td>
<td>0.0005</td>
<td>0.0004</td>
<td>6</td>
<td>0.0002</td>
<td>0.0001</td>
</tr>
<tr>
<td>Energy adjusted vitamin D 2010*</td>
<td>32</td>
<td>0.0005</td>
<td>0.0003</td>
<td>4</td>
<td>0.0003</td>
<td>0.0002</td>
</tr>
<tr>
<td>Energy adjusted calcium 2006b</td>
<td>35</td>
<td>0.1023</td>
<td>0.0261</td>
<td>6</td>
<td>0.1021</td>
<td>0.0257</td>
</tr>
<tr>
<td>Energy adjusted calcium 2010b</td>
<td>32</td>
<td>0.1221</td>
<td>0.0322</td>
<td>4</td>
<td>0.1129</td>
<td>0.0187</td>
</tr>
</tbody>
</table>

*Independent t-test; a=µg/kJ/d; b=mg/kJ/d

When only women who attended both years were included in the analysis, paired t-tests showed no significant difference in energy adjusted vitamin D intake in Caucasians (P=0.947) or Asians (P=0.926) between 2006 and 2010. For energy adjusted calcium intake, there was a significant difference between 2006 and 2010 in Caucasians (p<0.001) and in Asians (P=0.008). Therefore, energy adjusted calcium intakes, but not vitamin D intakes, varied by year in both ethnic groups.

3.8.7 Multiple regression analysis

To assess the predictors of between year change in 25(OH)D, two regression models were run. The first model contained age, change in BMI (2010-2006), ethnicity (Independent variables); and change in 25(OH)D (2006-10; dependent variable). The second model had the same parameters as model 1, but also added change in vitamin D and calcium intake, as well as change in mean weekly UVB exposure. It was intended to run model 2 including number of vitamin D producing holiday days as a predictor of change in 25(OH)D, but there were too few women in the sample with full data (n=9) if this was included in the model. Hence, it was deemed not valid to include this parameter in the model.

3.8.7.1 Model 1

In terms of model diagnostics, the model was a good fit to the data. Residuals were normally distributed with no evidence of heteroscedasticity. Standardized regression residuals showed a normal distribution. There was no significant collinearity between variables (tolerance 0.10; VIF<10). However, there was little predictive ability of these variables in explaining change in 25(OH)D (R²=0.08) (Table 3.9).
Table 3.9: Regression model 1 for prediction of change in Vitamin D status between 2006 and 2010 in a sample of postmenopausal women (n=36; 30 Caucasian and 6 Asian)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unstandardised coefficients</th>
<th>Standardised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
</tr>
<tr>
<td>Constant</td>
<td>-46.04</td>
<td>39.12</td>
</tr>
<tr>
<td>Age</td>
<td>0.67</td>
<td>0.60</td>
</tr>
<tr>
<td>Δ BMI</td>
<td>0.95</td>
<td>1.53</td>
</tr>
<tr>
<td>Ethnicity≠</td>
<td>1.32</td>
<td>0.96</td>
</tr>
</tbody>
</table>

ANOVA F=1.034, p=0.391

Explained variance $R^2=0.08$ (adjusted $R^2= 0.003$)

*Dependent variable=Change in 25(OH)D i.e. 25(OH)D2010 - 25(OH)D2006; ≠ dummy variable (Caucasian=0 and Asian=1). Δ BMI=change in BMI i.e. BMI2010- BMI2006.

The ANOVA F test showed that change in 25(OH)D was not predicted significantly by the model (F=1.034, P=0.391). Neither age (p=0.27), change in BMI (p=0.54), nor ethnicity (P=0.18) were significant predictors of change in 25(OH)D. The actual BMI change between 2006 and 2010 was very small (mean (SD) for all participants; 0.2 (2.3) kg/m$^2$), which could explain lack of predictive value for this variable. As was previously seen in Figure 3.4, actual change in 25(OH)D between 2006-2010 was very small with a mean (SD) change of 1.5 (14.2) nmol/L in the Caucasians and a reasonably small mean (SD) change of 6.4 (7.7) nmol/L in the Asians. This would explain the difficulty of the model parameters in predicting change in 25(OH)D.

3.8.7.2 Model 2

In terms of model diagnostics, model 2 was a good fit to the data. Standardised regression residuals showed a normal distribution with a random scatter of points on the standardised predicted vs. standardised residual scatterplot. There was no significant collinearity between variables. In terms of model results, model 2 was only a very small improvement on model 1, with $R^2=0.177$, (Table 3.10). Thus, there was still little predictive ability of these variables in explaining change in 25(OH)D. The ANOVA F test showed change in 25(OH)D was still not predicted significantly by the model (F=0.536, P=0.773). Neither age (p=0.39), change in BMI (P=0.22), ethnicity (P=0.86), change in vitamin D intake (P=0.76), change in calcium intake (P=0.57) or change in mean daily UVB exposure (P=0.33) were significant predictors of change in 25(OH)D.
Table 3.10: Regression Model 2 for prediction of change in Vitamin D status between 2006 and 2010 in a sample of postmenopausal women (n=22; 20 Caucasian and 2 Asian)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unstandardised</th>
<th>Standardised</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-64.8</td>
<td>68.07</td>
<td>-0.95</td>
<td>0.36</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.91</td>
<td>1.02</td>
<td>0.24</td>
<td>0.89</td>
</tr>
<tr>
<td>Δ BMI kg/m²</td>
<td>2.94</td>
<td>2.27</td>
<td>0.36</td>
<td>1.29</td>
</tr>
<tr>
<td>Ethnicity≠</td>
<td>2.31</td>
<td>12.49</td>
<td>0.05</td>
<td>0.19</td>
</tr>
<tr>
<td>Δ Vitamin D intake (µg/d)</td>
<td>-0.39</td>
<td>1.27</td>
<td>-0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>Δ Calcium intake (mg/d)</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.15</td>
<td>-0.58</td>
</tr>
<tr>
<td>Δ Mean UVB exposure (SED/day)</td>
<td>-3.81</td>
<td>3.77</td>
<td>-0.28</td>
<td>-1.01</td>
</tr>
</tbody>
</table>

ANOVA F=0.536, p=0.773  
Explained variance $R^2=0.177$ (Adjusted $R^2=-0.153$)

*Dependent variable=Change in 25(OH)D i.e. 25(OH)D2010 - 25(OH)D2006; ≠ dummy variable (Caucasian=0 and Asian=1). Δ BMI=change in BMI i.e. BMI2010- BMI2006; Δmean daily dietary vitamin D=dietary vitamin D 2010- dietary vitamin D 2006; Δmean daily dietary calcium=dietary calcium 2010- dietary calcium 2006; Δmean daily UVB exposure (SED)= mean daily UVB exposure 2010- mean daily UVB exposure 2006.

### 3.9 Discussion

#### 3.9.1 Vitamin D status, PTH, UVB exposure and diet: 2006 vs. 2010

This analysis has assessed data on change in vitamin D status between 2006 and 2010 in postmenopausal Asian and Caucasian women. It was found that in 2006 that Asians had lower vitamin D status than Caucasians, with half of Asian women being severely deficient (<25nmol/L), compared with none of the Caucasian women. The Asian women also had a higher PTH than Caucasian women. In contrast, in 2010 none of the Asian women were severely deficient and around 80% were insufficient in 25(OH)D (<50nmol/L). The equivalent figure for vitamin D insufficiency in Caucasians was 25%.

This analysis excluded women who had been on vitamin D supplements, suggesting that there has been some improvement in the vitamin D status of the Asian group, independent of supplement usage, since 2006. This could be due to increased sun exposure behavior, or it could be due to the differing vitamin D status of the women who returned in 2010 compared with those who did not. When looking at change in vitamin D status, for only women who attended both 2006 and 2010, there was still a trend for Asians to gain in vitamin D status by 6.4 nmol/L and for Caucasians to fall by 1.5 nmol/L. However, this ethnic difference in status over time was now not statistically
Vitamin D status

significant. The persistence of the improvement in status in the Asians when data are analysed within-subjects, albeit not statistically significant, suggests that it may be a change in behavior of the groups, probably due to increased awareness of vitamin D deficiency, rather than the inclusion of different women in different years that is most likely to explain the improved vitamin D status in Asian women in 2010. It is unfortunate that no information is available on the change in dietary intakes of vitamin D in Asians who had taken part in the study in 2006, but did not return in 2010.

There are no between year data on 25(OH)D status in South Asian populations to compare our data with. However, our Caucasian estimates (a stable 25(OH)D concentration) are lower than that seen in the two USA studies (-10 nmol/L) (Looker et al. 2008) and (-6nmol/L) (Ginde, Liu, and Camargo 2009) and the Canadian study (+4.7 nmol/L)(Berger et al. 2012). However, our findings are similar to that of the Norwegian study of Jorde et al. (2010) who found that most subjects tracked within their 25(OH)D quartile over a long time period (14 years) (Jorde et al. 2010). A similar result was found in our analysis, whereby paired data showed there were no differences between participant vitamin D status quartiles between 2006 and 2010 (in either ethnic group). Between study differences are likely due to differences in the timescales and populations studied, the different countries’ policy to vitamin D fortification and supplementation, different baseline 25(OH)D concentration, whether supplement users were included in the analysis and whether standardised for age, as well as differing sun exposure and dietary behavior between populations.

There are no previous studies assessing between year changes in vitamin D status in South Asian groups. The per cent change in persons classified as severely deficient rose from 15 to 19% in the 3 year period between the last two NDNS surveys (2008/9 - 2010/11) (DOH 2011). In our 4 year time period (2006-2010), Asians dropped from 50% to 0% under 25nmol/L, but Caucasians remained stable at 0% under 25nmol/L. Therefore, our South Asian women had considerably higher change in 25(OH)D status than that seen in the NDNS for the whole UK population (DOH 2011). Interestingly, our Caucasian population showed less change than the NDNS, with no change seen at all in % of women under 25nmol/L. The discrepancy between these results and the NDNS is likely due to the unique nature of the groups being studied in this research. This includes the participation of the Asian women in the D-FINES study in 2006, and the fact that the Caucasian women who took part had a very good initial vitamin D status in 2006. Hence, in this study no change in vitamin D status in Caucasians has been seen over time, unlike other studies. The limitations of the participant sample will be discussed further in Chapter 7 (Section 7.4.2).

In 2006, there was an ethnic difference in daily UVB exposure (SED), with Caucasians having a higher UVB exposure than all Asians. However, in 2010 this difference had disappeared. In terms of within group change from 2006 to 2010, only Caucasians showed a significant change in UVB
Vitamin D status

exposure, with a drop from 1.4 SED per day to 0.4 SED per day. Part of this difference in UVB exposure is likely to be due to the fact that summer 2010 was a ‘poor summer’, with the coolest August since 1993 (Metoffice 2010). In contrast, summer 2006 was a very hot summer, with many UK areas in July and September having the hottest recorded temperatures for their respective months since 1914 (Metoffice 2006). Without sun exposure records completed by the participants it is not possible to quantify how much of the reduced sun exposure was due to changes in weather conditions, and how much was due to weather independent changes in participant behavior. Written diary records had been used in 2006, but were not found to be a reliable indicator of sun behavior, likely due to low participant compliance. Consequently, these sun exposure diaries were not used again in 2010.

In paired analyses, the trend for an increase in sun exposure of the Asian women from a mean of 0.3SED per day in 2006, to a mean of 0.4SED per day in 2010 is an interesting finding. This represents an increase of 33% in the Asian group from 2006, despite less sunshine availability in 2010. This increase in UVB may partly explain the improved vitamin D status in 2010 as compared with 2006 in the Asian group. It is unclear whether the less intense sunlight in 2010 encouraged the Asian women to go outdoors, or whether there was a conscious attempt in the Asian group to increase sun exposure since participation in the 2006 study. Unpaired analyses, showed an ethnic difference in UVB in 2006, but not in 2010. This was due to the falling UVB exposure of the Caucasian group, and the rising UVB exposure of the Asian group reported above. Vitamin D levels remained stable in the Caucasian group, with Caucasians having a higher vitamin D status than Asians in both 2006 and 2010. The fact that Caucasian vitamin D status stayed stable between years, even though UVB decreased, can be explained by the fact that their vitamin D status was high, and therefore extra UVB was not necessarily translating into higher vitamin D status (due to the regulatory mechanisms in skin to stop over production of vitamin D). As described above, there was improvement in the vitamin D status of the Asian women in 2010 as compared with 2006. This is likely due to their higher UVB exposure, which was likely to have had an effect on increasing vitamin D levels in this group as they were initially low in 2006.

It must also be borne in mind that it is likely that the UVB analysis is underpowered, due to small numbers of women in the Asian group. This may explain why the reduction in UVB exposure in the paired Asian data of 33% did not reach statistical significance. A retrospective power calculation for this analysis suggested a power of 10.6% for a statistical significance level of 5%. Moreover, n=41 Asians in both 2006 and 2010 would be required for 80% power\(^5\). Despite this however, it remains

\(^5\) Calculations were based on the assumption of independent samples. Therefore, these estimates are conservative because statistically, less subjects are required for paired data than for independent samples data for equal power.
an interesting finding which warrants further investigation. There are no previous studies assessing change in UVB exposure over a period of years in either South Asians or Caucasians and the data presented here is novel. Again, it is difficult to generalise from these results to the general population of Asian women due to the problem of a possible change in behavior due to the influence of the women learning about vitamin D during the original study.

There was no ethnic difference in reported calcium intake in either 2006 or 2010. Both groups had a sufficient calcium intake, at around 700-800g/d in 2006 and 800-875g/d in 2010. For vitamin D intake, there was only a significant ethnic difference for 2006 only, with Asians having an average intake of 1.9 micrograms per day, compared with 3.1 micrograms per day in Caucasians. In 2010, intakes had equalised, with 2.3 micrograms per day in Asians and 2.6 micrograms per day in Caucasians. This suggests a possible change in the Asian diet. Further analysis of the individual food items contributing to vitamin D status would be required to address the likelihood of this being the case. However, there may be another explanation. When only women who had attended both 2006 and 2010 were included in the results the significant ethnic difference in vitamin D intake in 2006 disappeared, and Asian intakes changed to 2.2 (0.5) μg/d in 2006 and 2.2 (1.6) μg/d in 2010. The equivalent values for Caucasians were now 3.5 (3.0) μg/d in 2006 and 2.9 (1.6) μg/d in 2010. So the lack of ethnic difference in 2006 when using paired data is likely to be due to a real lack of difference, rather than just by a reduction in subject numbers (and thus statistical power). Alternatively, it may be that when just comparing the women who came both times, some of the women who attended only in 2006 are removed. These women may be contributing to the ethnic difference that is seen in vitamin D intake when all women are included, regardless of number of visits. When only comparing women who came both times, the Asian dietary intakes of vitamin D became equal at around 2.2 micrograms per day in both 2006 and 2010, suggesting a change in Asian diet is unlikely. This contradicts other studies which have found that Vitamin D intake is lower in western dwelling South Asian children (Donin et al. 2010) and adults (Wu et al. 2009) than in same age UK Caucasians.

3.9.2 Predictors of between year change in 25(OH)D- regression analysis

Looking at change in 25(OH)D between the two years, vitamin D status in the Caucasian group fell on average by 1.5nmol/L from 2006 to 2010, whereas in Asians it increased by 6.4 nmol/L in the same time period. Regression models were not able to predict this change in 25(OH)D, using age, ethnicity and change in BMI (2006 to 2010) as parameters. Age (Freedman et al. 2013), ethnicity (Darling et al. 2013) and BMI (Tran et al. 2013) are known to be predictors of vitamin D status. Adding dietary vitamin D and calcium, as well as UVB exposure to the model did not improve predictive ability. This is surprising considering UVB is known to be the main contributor to
Vitamin D status

vitamin D status. The most probable explanation for the lack of predictive ability of the model was that the change in 25(OH)D was too small, and perhaps not variable enough between participants. This is more likely than the parameters not being influential on 25(OH)D status per se. Alternatively, the dosimeter badge readings, 25(OH)D measurements and food diary estimates may not be providing an accurate picture of the participants’ general diet and sunlight exposure; meaning they do not predict 25(OH)D concentration as well as would be expected. In the Berger et al. (2012) CaMos study, change in 25(OH)D over time was positively associated with age, higher dietary calcium, lower BMI and increased sunlight exposure (Berger et al. 2012). Similarly, in the Jorde et al. (2010) study, the predictors of change in 25(OH)D were changes in dietary vitamin D, body weight and physical activity (Jorde et al. 2010). In the Looker et al. (2008) study, changes in milk intake, usage of sun protection and BMI were associated with changes in 25(OH)D status (Looker et al. 2008). Of note, the Asian women in the regression analysis had a higher socio-economic status, were older and had a lower BMI than the whole 2010 postmenopausal cohort. This suggests the women who were eligible to enter the regression model (i.e. had complete data for all model parameters) were in some ways different to that of the cohort they were drawn from. Had the model been statistically significant, this would have had implications for the interpretation of the results.

3.9.3 Limitations

As mentioned above, the small number of subjects in the Asian group limits the power for the analyses in this chapter, in comparison to that of the Caucasian group. There may have been some change in participant behaviour between the two studies, as a result of taking part in the 2006 study, which may cause the results of this study to differ from the larger population surveys. The women studied vary from the general population, and are perhaps unusual in their taking part in a research study. Research participants who have attended previous measurements for a study on vitamin D are likely to be better informed about vitamin D deficiency and its consequences than the general population. This may have led to some of the change in vitamin D status, UVB exposure or diet that we saw between 2006 and 2010. The problem of increased knowledge of cohort study participants was also raised by Berger et al. (2012) in their discussion of their Canadian CaMos study results (Berger et al. 2012).

There are known limitations to the use of food diaries and dosimeter badges, to collect diet and UVB data respectively (see chapter 2, Section 2.3.7 for a discussion of these limitations). Also, there are limitations to the use of immunoassays, rather than HPLC, for measurement of 25(OH)D concentration (see chapter 2, Section 2.3.1 for a discussion of these limitations). Last, there are difficulties in accurately assessing whether holiday days are likely to be ‘vitamin D producing’ or
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not. Even in the best scenario, this is likely to be a crude measure, considering the complexity of vitamin D production and its multiple contributory factors (e.g. individual skin type, time of day, weather, clothing, sun protection, participant behaviour), as well as the self-reported nature of the data. However, despite this, this data gives a picture of the pattern of changes in vitamin D status and its contributing factors in a group of South Asian women, information which is lacking in the literature base.

3.10 Conclusion

Asians had a lower vitamin D status in 2006 than Caucasians, with severe deficiency being very common in Asians. However, vitamin D status in Asians had improved in 2010, with no women being severely deficient in either ethnic group. There was a trend for daily UVB exposure to increase in Asians between 2006 and 2010, but not in Caucasians, where it fell from 2006-2010. These differences are most likely to be due to differences in weather patterns and individual behaviour between years. There were no ethnic differences in calcium intake in either 2006 or 2010, and only an ethnic difference in vitamin D intake in 2006. Age, ethnicity, change in BMI, change in dietary vitamin D and calcium, and change in mean daily UVB exposure were not able to predict change in 25(OH)D between 2006 and 2010. This is likely due to the very small change in 25(OH)D seen over this period, and possible lack of inter-individual variation in 25(OH)D, rather than the above factors being truly non-contributory to 25(OH)D status. However, this data suggests interesting trends which warrant further investigation.
CHAPTER 4- Musculoskeletal health and vitamin D status
Part I: Skeletal health– ethnic differences and associations with vitamin D status
n.b. This part of Chapter 4 has been published:

See Appendix II- Publications and conference abstracts for full paper

4.1 Introduction

There is some concern that migrant Asians to western societies may have poorer musculoskeletal health than the indigenous population. For example, a recent U.S. study found that South Asian women had a higher prevalence than Caucasian women of femoral neck osteoporosis (Khandewal et al. 2012). South Asian women have also been found to have a higher incidence of wrist fracture than Chinese women, although South Asians did not differ from White Caucasian women on these measures (Khandewal et al. 2012; Lofthus et al. 2008). To investigate these epidemiological findings of increased osteoporosis incidence, but similar fracture rates, it is important to examine the differences in bone geometry between women of South Asian and White ethnicity.

Research studies generally suggest a lower areal bone mineral density (aBMD) in South Asian compared with Caucasian women (Hamson et al. 2003; Brooke-Wavell et al. 2008). However, aBMD measures do not assess true volumetric density (vBMD), as they are influenced by actual bone size. The smaller bone size of South Asian populations has been found to explain the apparent lower aBMD in this ethnic group as compared with Caucasians (Cundy et al. 1995; Roy et al. 2005). Hence, to confirm if South Asians do have a lower bone density than Caucasians, it is important that their volumetric bone mineral densities (vBMD) are investigated in addition to bone structure (e.g. cortical and trabecular density) and size. The known small bone size in South Asians suggests a biomechanical detriment that is likely to lead to increased risk of fracture. Two studies (Roy et al. 2005; Ward et al. 2007) suggest that premenopausal South Asian women in the UK have smaller bone size and similar or lower vBMD to Caucasian women. However, there is no equivalent published data for postmenopausal South Asian women to date. Recently, bone geometry in Chinese women has been examined in detail using HR-pQCT, and it has been found that an increased vBMD and cortical thickness, when compared to Caucasians, may lead to increased bone strength, despite a smaller bone size [9, 15-17](Walker et al. 2011; Walker et al. 2009; Walker et al. 2012). It would be interesting to assess whether similar adaptations are seen in South Asian women.

Few studies have assessed the relationship between vitamin D status and bone geometry using pQCT methods. The few studies that have been done have found a relationship between vitamin D status
and tibial cortical bone mass in children (Sayers et al. 2012) and increased tibial cortical vBMD (Pedone et al. 2010), radial cortical vBMD (Viljakainen et al. 2010) and radial total bone density (Boonen et al. 1997) in adults. Also, studies of maternal vitamin D status have found that in infants born to mothers with higher vitamin D status have increased tibial bone size and BMC (Viljakainen et al. 2010). Nonetheless, no research to date has assessed whether there are ethnic differences in the association between vitamin D status and bone geometry, including no research assessing South Asian populations. Due to possible ethnic differences in genetics and calcium metabolism it is possible that the association between vitamin D status and bone geometry varies by ethnicity, therefore it is important that the relationship is assessed in different ethnic groups.

The aim of this study was to assess bone geometry at the radius and tibia, using pQCT, in postmenopausal South Asian women and compared them with postmenopausal Caucasian women. Also, the study aimed to assess whether 25(OH)D is associated with bone geometric measures. Based on previous research comparing South Asian and White Caucasian populations (Roy et al. 2005), it was hypothesised that for all sites of the radius, total vBMD would be similar between the two ethnic groups, but with South Asians having a smaller bone size. Previous analysis of this dataset has reported that the postmenopausal South Asian women have a reduced standing height and increased relative body weight for height compared with postmenopausal Caucasians (Macdonald et al. 2011). It was hypothesised that there would be increased tibial vBMD in South Asians compared to Caucasians, as a result of the increased weight bearing load of a larger BMI on a smaller bone size. It was also predicted that 25(OH)D concentration would be positively associated with cortical, trabecular and total bone density, in both ethnic groups.

4.2 Methods

4.2.1 Participants undergoing pQCT analysis

Details of participant recruitment and inclusion-exclusion criteria for the 2010 cohort were presented previously in Chapter 2, Section 2.1. Of the women participating in the 2010 measurements, n=82 (n=21 South Asian; n=61 Caucasian) were postmenopausal and their results are reported here. All of these n=82 postmenopausal women had a pQCT scan of the radius undertaken. Due to time constraints an opportunistic sub-sample, n=76, also had the tibia scanned (n=19 South Asian; n=57 Caucasian).

Some of the women, as a result of the DXA scan obtained during the original study in 2006, had subsequently been diagnosed with osteoporosis or osteopenia and were now on anti-resorptive medications (mainly bisphosphonates). These women on bone medications (n=14; n=3 South Asian;
n=11 Caucasian) had pQCT scans undertaken but were excluded from the current data analysis. This left n=68 (n=50 Caucasian and n=18 South Asian) radius measurements and n=65 (n=48 Caucasian and n=17 South Asian) tibia measurements for use in the current analysis. Anthropometric and demographic information (height, weight, hip and waist circumference) were collected as described previously (Chapter 2, Section 2.1).

4.2.2 Bone densitometry

Bone geometry was measured at the radius and tibia using a Stratec X2000L (Stratec Medizintechnik, GmbH, Pforzheim, Germany; software version 6.20) pQCT scanner. The radius in the non-dominant arm and the equivalent tibia were scanned. Radius slices (2.2mm) were taken at the distal end and at the mid shaft of the radius (4% and 66% sites). Tibia slices (2.2mm) were taken at the distal end of the tibia shaft (4%) and also further up the tibial shaft (14% and 38% sites). Figure 4.1 illustrates these scan positions, with the parameters measured or calculated at each site. The distal radius (4%) was examined due to the clinical significance of this site. One diaphyseal radius site (66%) and two diaphyseal sites of the tibia (14% and 38%) were also measured due to the potential importance of considering the morphology of the whole bone for fracture risk.

![Figure 4.1: Scan sites used for Tibia and Radius](image)

Equal numbers of South Asian and Caucasian subjects were scanned by each of the two operators of the machine (ALD and OAH). Scanning procedure for both the South Asian and Caucasian women was also identical, using the same pQCT machine and software with standardised instructions. Particularly, the procedure for measuring the length of radius and tibia was standardised and undertaken as per manufacturer’s guidelines, Hence, radial object length was assessed as the distance (in mm) from the processus styloideus to the olecranon. Tibial object length was assessed as the distance from the middle of the inner ankle to the tibial plateau. A scout view of 30 lines, at 40mm/sec was run for each participant for each scan. The reference line was placed at the cortical end plate of the radius or distal end of the tibia, as appropriate. The pQCT scan was run at 20mm/sec.
for the tibia and 30mm/s for the radius, both with a voxel size of 0.50mm. For analysis, the threshold for cortical bone was set automatically by the software at 711mg/cm$^3$. All standard parameters (vBMD, BMC, bone area, bone density of trabecular and cortical areas, cortical thickness and periosteal/endosteal circumferences) were reported.

The polar strength-strain index (SSI$p$) is a measure of the bone’s ability to resist torsional forces, and was calculated automatically by the software using the formula

$$SSI = \sum_{i=1,n}((\tau_i^{2}\pi a^{3}CD)/r_{max}^{3}*ND).$$

$CD=$ measured cortical density (mg/cm$^2$) and $ND=$ normal physiological density (1200mg/cm$^3$) (source: Stratec manual 6/11/9 Man62e.doc)

Fracture load (under bending) was calculated by the software using a bending test length of 180mm and bone ultimate bending strength of 180Mpa. The formula used by the Stratec software for calculation of bending fracture load is:

$$F_B = 4\sigma_B * SSI$$

$$F_B=$$ Fracture load [N]; $\sigma_B=$ Ultimate load = 180 Mpa; $l=$ distance between supports

### 4.2.3 Statistical Analysis

For unadjusted analyses, independent t-tests were performed to assess ethnic differences in each available pQCT parameter at each site. Analysis of Covariance (ANCOVA) was then used to adjust for age, height and BMI in separate analyses. These confounders were not assessed together due to the high degree of correlation between them. To compare the results at each bone site (within-subjects) repeated measures analysis of variance (ANOVA) (for parameters measured at three sites) and paired t-tests (two sites) were used.

Pearsons and partial correlations were used to assess the relationship between 25(OH)D and the pQCT indices. The partial correlations were corrected for potential confounders (age and BMI). Age and BMI were not corrected for in the same analysis due to inter correlation between these variables. This analysis excluded women on bone medications, but included those on vitamin D supplements. The 25(OH)D data used in this analysis was as measured by HPLC.

All variables not showing a normal distribution were log transformed prior to statistical analysis, and normality re-checked by Kolmogorov-Smirnov test. PASW (v.18.0) (Chicago, IL) was used for the t-test, ANOVA and correlational statistical analyses. Graph Pad Prism (v.5.04) (San Diego, CA) was used for the linear regression analyses and production of figures. Statistical significance was assessed using the conventional $p \leq 0.05$. 

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4.3 Results

4.3.1 Participant characteristics for sub-analyses

Participant characteristics for each of the different sub-analyses in Sections 4.3.2 to 4.3.5 are given in Tables 4.1 to 4.4. Assessing the data for all women with radius measurements (Table 4.1), South Asian women had a significantly higher BMI compared to the Caucasian women (P=0.007), with the Asians on average being classified as borderline overweight-obese (mean=29.6, SD=4.2), and the Caucasians on average being considered borderline normal-overweight (mean=25.9, SD=5.0). There was a small but significant difference in age between the two groups, with Caucasians on average two years older than Asians (66 ± 4.8) vs. (64 ± 3.6) years respectively (P=0.05) but no significant difference in years since onset of menopause. Last, dietary calcium intake (P=0.018) and 25(OH)D status were significantly higher in the Caucasians (P<0.001).

Tables 4.1 to 4.4 suggest that mean and SD for height, weight, BMI, age and time from menopause were similar for all the sub-analyses. This gave confidence that the small amount of missing data for some parameters for some women in each analysis was not deviating from the general characteristics of the sample as a whole. See Appendix AA for characteristics of the whole postmenopausal 2010 cohort (n=82).

Table 4.1: Participant characteristics- subsample of postmenopausal women with radius measurements (n=68)

<table>
<thead>
<tr>
<th></th>
<th>CAUCASIAN n=50</th>
<th>ASIAN n=18</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>Mean 161.8 SD 6.8</td>
<td>Mean 154.6 SD 4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.9 12.0</td>
<td>69.6 9.2</td>
<td>0.594</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 5.0</td>
<td>29.6 4.2</td>
<td>0.007</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.9 4.8</td>
<td>63.5 3.6</td>
<td>0.052</td>
</tr>
<tr>
<td>Time since menopause (years)</td>
<td>16.3 6.7</td>
<td>16.8 2.9</td>
<td>0.861</td>
</tr>
<tr>
<td>Vitamin D intake (µg/d)</td>
<td>2.7 1.9</td>
<td>1.8 1.8</td>
<td>0.170</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>779 237</td>
<td>579 216</td>
<td>0.018</td>
</tr>
<tr>
<td>25(OH)D nmol/L ≠</td>
<td>82.3 19.3</td>
<td>58.9 22.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTH pmol/L</td>
<td>2.4 1.0</td>
<td>2.6 0.8</td>
<td>0.557</td>
</tr>
</tbody>
</table>

a Age ranges: Asians=58-71 years; Caucasians 59-75 years ≠measured by HPLC
### Table 4.2: Participant characteristics- subsample of postmenopausal women with tibia measurements (n=65)

<table>
<thead>
<tr>
<th></th>
<th>CAUCASIAN n=48</th>
<th>ASIAN n=17</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
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<tr>
<td>Weight (kg)</td>
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<td>70.6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>26.0</td>
<td>5.0</td>
<td>29.5</td>
</tr>
<tr>
<td>Age (years)a</td>
<td>66.0</td>
<td>4.8</td>
<td>63.6</td>
</tr>
<tr>
<td>Time since menopause (years)</td>
<td>16.3</td>
<td>6.8</td>
<td>16.8</td>
</tr>
<tr>
<td>Vitamin D intake (µg/d)</td>
<td>2.8</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>783.5</td>
<td>237.8</td>
<td>578.9</td>
</tr>
<tr>
<td>25(OH)D nmol/L≠</td>
<td>81.7</td>
<td>18.9</td>
<td>59.3</td>
</tr>
<tr>
<td>PTH pmol/L</td>
<td>2.4</td>
<td>0.8</td>
<td>2.6</td>
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a Age ranges: Asians=58-71 years; Caucasians=59-75 years ≠measured by HPLC

### Table 4.3: Participant characteristics- women with 25(OH)D and radius measurements (n=62)

<table>
<thead>
<tr>
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<th>ASIAN n=17</th>
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<tr>
<td>Height (cm)</td>
<td>162.0</td>
<td>6.4</td>
<td>154.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.5</td>
<td>10.7</td>
<td>69.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4</td>
<td>4.5</td>
<td>28.9</td>
</tr>
<tr>
<td>Age (years)a</td>
<td>66.0</td>
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<td>63.4</td>
</tr>
<tr>
<td>Time since menopause (years)</td>
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<td>16.3</td>
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<td>Vitamin D intake (µg/d)</td>
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<td>1.8</td>
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<tr>
<td>Calcium intake (mg/d)</td>
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<td>243.7</td>
<td>578.9</td>
</tr>
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<td>25(OH)D nmol/L≠</td>
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<td>19.3</td>
<td>58.9</td>
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<tr>
<td>PTH pmol/L</td>
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<td>1.0</td>
<td>2.6</td>
</tr>
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</table>

a Age ranges: Asians=58-71 years; Caucasians=59-75 years ≠measured by HPLC

### Table 4.4: Participant characteristics- women with 25(OH)D and tibia measurements (n=59)

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<td>6.4</td>
<td>155.0</td>
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<tr>
<td>Weight (kg)</td>
<td>67.0</td>
<td>10.6</td>
<td>69.3</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.6</td>
<td>4.6</td>
<td>28.8</td>
</tr>
<tr>
<td>Age (years)a</td>
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<td>Vitamin D intake (µg/d)</td>
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<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>787.7</td>
<td>243.9</td>
<td>578.9</td>
</tr>
<tr>
<td>25(OH)D nmol/L≠</td>
<td>81.7</td>
<td>18.9</td>
<td>59.3</td>
</tr>
<tr>
<td>PTH pmol/L</td>
<td>2.4</td>
<td>0.8</td>
<td>2.6</td>
</tr>
</tbody>
</table>

a Age ranges: Asians=58-71 years; Caucasians=59-75 years ≠measured by HPLC

### 4.3.2 Ethnic differences in bone parameters

In the text, for brevity, unadjusted data is reported, with the adjustments also reported if deemed to be significant to the interpretation of the results.
4.3.2.1 Distal radius- 4%

A summary of the radial bone parameters can be seen in Table 4.5. There was no significant difference in distal radial total BMC by ethnicity, even after adjustment for confounders. Asians had a significantly smaller area than Caucasians (-18%, p<0.001) and a significantly greater total density (+13%, P=0.014). Trabecular area was significantly smaller in Asians than Caucasians (-18%, P<0.001), but trabecular density was not significantly different (Figure 4.2).

Table 4.5: Radial bone geometry outcomes by ethnic group- raw and adjusted data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CAUCASIAN n=50</th>
<th>ASIAN n=18</th>
<th>Independent t-test/ANCOVA</th>
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</thead>
<tbody>
<tr>
<td>Radius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% Radius BMC g/cm</td>
<td>1.03 (0.20)</td>
<td>0.96 (0.18)</td>
<td>93.2 (0.203, 0.176, 0.997, 0.075)</td>
</tr>
<tr>
<td>ToA mm²</td>
<td>384 (63)</td>
<td>314 (45)</td>
<td>81.8 (&lt;0.001, 0.000, 0.043, &lt;0.001)</td>
</tr>
<tr>
<td>ToD mg/cm³</td>
<td>272 (54)</td>
<td>306 (35)</td>
<td>112.8 (0.014, 0.027, 0.089, 0.100)</td>
</tr>
<tr>
<td>Trab A mm²</td>
<td>173 (28)</td>
<td>141 (20)</td>
<td>81.8 (&lt;0.001, 0.000, 0.043, &lt;0.001)</td>
</tr>
<tr>
<td>Trab D mg/cm³</td>
<td>168 (44)</td>
<td>183 (33)</td>
<td>108.6 (0.209, 0.193, 0.314, 0.524)</td>
</tr>
<tr>
<td>66% Radius BMC g/cm</td>
<td>0.99 (0.20)</td>
<td>0.88 (0.15)</td>
<td>88.9 (0.059, 0.029, 0.152, 0.001)</td>
</tr>
<tr>
<td>ToA ≠ mm²</td>
<td>154 (38)</td>
<td>130 (26)</td>
<td>84.6 (0.039, 0.038, 0.068, 0.002)</td>
</tr>
<tr>
<td>ToD mg/cm³</td>
<td>655 (99)</td>
<td>688 (82)</td>
<td>105.1 (0.209, 0.396, 0.084, 0.178)</td>
</tr>
<tr>
<td>SSIp ≠ mm³</td>
<td>245 (79)</td>
<td>196 (57)</td>
<td>79.9 (0.027, 0.022, 0.185, 0.002)</td>
</tr>
<tr>
<td>CoA mm²</td>
<td>68 (16)</td>
<td>61 (11)</td>
<td>89.7 (0.042, 0.024, 0.415, 0.007)</td>
</tr>
<tr>
<td>CoD ≠ mg/cm³</td>
<td>1070 (51)</td>
<td>1088 (40)</td>
<td>101.7 (0.179, 0.533, 0.004, 0.113)</td>
</tr>
<tr>
<td>CT mm</td>
<td>1.8 (0.4)</td>
<td>1.8 (0.4)</td>
<td>97.8 (0.73, 0.359, 0.468, 0.512)</td>
</tr>
<tr>
<td>CT:ToA</td>
<td>1.8:15</td>
<td>1.8:130</td>
<td>-</td>
</tr>
<tr>
<td>PC mm</td>
<td>43.6 (5.6)</td>
<td>40.3 (3.9)</td>
<td>92.3 (0.024, 0.023, 0.035, 0.001)</td>
</tr>
<tr>
<td>EC mm</td>
<td>32.2 (6.6)</td>
<td>29.1 (5.0)</td>
<td>90.4 (0.077, 0.108, 0.034, 0.009)</td>
</tr>
</tbody>
</table>

Radius site 4% (distal) 66% (mid shaft) BMC= bone mineral content SSIp=polar strength strain index, ToA= total area, ToD=total density, CoA=cortical area, CoD=cortical density, PC=periosteal circumference, EC=endosteal circumference.

TrabA=trabecular area, TrabD=trabecular density “Raw data; “adjusted for age ‘adjusted for height ‘adjusted for BMI ; ≠log transformed for statistical analysis.

4.3.2.2 Radial Shaft-66%

Total area (-15%, P=0.039) and age adjusted total BMC (-12%, P=0.029) were significantly smaller in the Asians. Cortical area was also smaller in Asians (-10%, P=0.042), but there was no significant ethnic difference in cortical density. There was also no significant ethnic difference in cortical
thickness, suggesting that this was thicker in Asians in proportion to their smaller overall bone size. In terms of bone strength, SSIp was significantly higher by around 20% in Caucasians (P=0.023). This difference in SSI was not statistically significant when height was controlled for, suggesting it was due to the smaller skeletal size of the Asians.

Figure 4.2: Radial bone geometry, unadjusted data Asian as a per cent of Caucasian values (n=18 Asian; n=50 Caucasian)

4.3.2.3 Distal Tibia- 4%

A summary of the tibia bone parameters can be seen in Table 4.6. There was no significant ethnic difference in total BMC, but total area was significantly smaller in Asians (-16%, P=0.005; Figure 4.3). Accordingly, total density was significantly larger (+12%, P=0.003). The increased total density did not remain significant when BMI was controlled for, suggesting this might be influenced by the increased weight for height in the Asians. Trabecular area was larger in Caucasians (+16%, P=0.005); but there was no significant ethnic difference in trabecular density.

4.3.2.4 Tibia Shaft-14%

BMC was significantly lower in the Asians (-24%, P=0.013), with total area smaller by 27% (P=0.002) and total density higher by 29% (P<0.001). Also, cortical area was significantly smaller by 19% in Asians (P=0.051), with cortical density 5% higher (P=0.001). The increased cortical density was still significant after adjusting for the confounders, suggesting this was not due to increased BMI. There was a 7% increased cortical thickness in the Asians group, but this was not statistically significant. For bone strength, SSIp was significantly reduced in Asians by 37% (p=0.006). These differences remained significant, even after adjusting for age, height and BMI (P<0.01).
4.3.2.5 Tibia Shaft-38%

Total bone area was smaller in Asians (-30%, P=0.003) with a smaller BMC (-27%, P=0.004). There was no significant difference in total density, or cortical density by ethnicity. Cortical area was significantly smaller by 28% (P=0.004) and cortical thickness was significantly smaller by 17% (P=0.035). This suggested a cortical thickness that is proportionately larger in Asians (17% smaller than Caucasians) than would be expected for their overall smaller bone size (30% smaller than Caucasians). In Asians, SSIp was significantly reduced by 38% (P<0.001).
Table 4.6: Tibial bone geometry outcomes by ethnic group- raw data and adjusted for age/ height/ BMI

<table>
<thead>
<tr>
<th></th>
<th>CAUCASIAN (C) n=48</th>
<th>ASIAN (A) n=17</th>
<th>Independent t-test/ANCOVA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>4% Tibia BMC g/cm</td>
<td>3.02</td>
<td>0.76</td>
<td>2.87</td>
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<tr>
<td>ToA mm^2</td>
<td>1116</td>
<td>238</td>
<td>940</td>
</tr>
<tr>
<td>ToD mg/cm^3</td>
<td>272</td>
<td>49</td>
<td>304</td>
</tr>
<tr>
<td>TrabA mm^2</td>
<td>502</td>
<td>107</td>
<td>423</td>
</tr>
<tr>
<td>TrabD mg/cm^3</td>
<td>226</td>
<td>48</td>
<td>237</td>
</tr>
<tr>
<td>14% Tibia BMC g/cm</td>
<td>2.26</td>
<td>0.35</td>
<td>1.71</td>
</tr>
<tr>
<td>SSIP mm^3</td>
<td>1366</td>
<td>291</td>
<td>860</td>
</tr>
<tr>
<td>ToA mm^2</td>
<td>505</td>
<td>84</td>
<td>316</td>
</tr>
<tr>
<td>ToD mg/cm^3</td>
<td>457</td>
<td>84</td>
<td>587</td>
</tr>
<tr>
<td>CoA mm^2</td>
<td>140</td>
<td>27</td>
<td>116</td>
</tr>
<tr>
<td>CoD mg/cm^3</td>
<td>1033</td>
<td>52</td>
<td>1084</td>
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<tr>
<td>CT mm</td>
<td>1.9</td>
<td>0.4</td>
<td>2.1</td>
</tr>
<tr>
<td>38% Tibia BMC g/cm</td>
<td>3.21</td>
<td>0.40</td>
<td>2.34</td>
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<tr>
<td>SSIP mm^3</td>
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<td>283</td>
<td>905</td>
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<td>ToA mm^2</td>
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<td>ToD mg/cm^3</td>
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<td>840</td>
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<td>CoA mm^2</td>
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<td>183</td>
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<tr>
<td>CoD mg/cm^3</td>
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<td>35</td>
<td>1151</td>
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<td>-</td>
<td>3.8:272</td>
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<td>79.4</td>
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<tr>
<td>EC ≠ mm</td>
<td>67.2</td>
<td>8.1</td>
<td>47.3</td>
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Tibia site 4% (distal) 14% (shaft) 38% (shaft) BMC= bone mineral content, SSIP=polar strength strain index, ToA= total area, ToD=total density, CoA=cortical area, CoD=cortical density, PC=periosteal circumference, EC=endosteal circumference, TrabA=trabecular area, TrabD=trabecular density “Raw data; ^adjusted for age ‘adjusted for height ^adjusted for BMI ; ≠log transformed for statistical analysis
Figure 4.3: Tibial bone geometry, unadjusted data Asian as a per cent of Caucasian values (n=17 Asian; n=48 Caucasian) Mass= Bone mass, SSIp=polar strength strain index, SSIx and SSIy- strength strain index with respect to x and y axes ToA= total area, ToD=total density, CoA=cortical area, CoD-cortical density, CT=cortical thickness, PC=periosteal circumference, EC=endosteal circumference, TrabA=trabecular area, TrabD=trabecular density. Bone geometry, unadjusted data Asian as a percent of Caucasian values. P values derived from Independent t-test.

4.3.3 Differences by site within ethnicity

The unadjusted data for parameters with only two measurements are illustrated in Figure 4.4. From the distal radius to the mid shaft radius, in both ethnic groups, BMC and total area decreased, but the decrease in area was greater in Caucasians and the decrease in BMC was greater in Asians. BMC differences between the two sites were not statistically significant in Caucasians (P=0.09), but were for Asians (P=0.02), and area differences were significant in both groups (P<0.001). Also, the total density rose slightly but significantly between the two sites (P<0.001) for both groups.
Musculoskeletal Health and Vitamin D status

Figure 4.4: Geometry outcomes by site moving up the radial shaft length - unadjusted data (n=18 Asian; n=50 Caucasian) BMC = bone mineral content

For the tibia, as expected from the higher torsion and bending forces acting on the mid shaft, both groups showed a rise inSSIp and SSIX from 14% tibia to 38% tibia (Figure 4.5). However, this rise was only statistically significant in Caucasians (SSIp P=0.006; SSIX P<0.001). In both ethnic groups SSIIy showed a very small change, which was statistically significant in Caucasians only (P=0.009). Cortical density and cortical area both showed a sharp increase from 14% tibia to 38% tibia, especially in Caucasians (P<0.001), compared to an attenuated but still significant increase in Asians (P<0.001). Cortical thickness rose significantly (P<0.001) in both groups. Concurrently, there was a significant reduction in endosteal (P<0.001), but not periosteal (P=0.188) circumference in Asians. Both endocortical and periosteal circumferences became significantly smaller in Caucasians from 14% tibia to 38% tibia, (P<0.001), suggesting an even thicker cortex in an even smaller bone.

For the tibia only, Figure 4.6 illustrates the data for parameters that had three measurements. All repeated measures comparisons were statistically significant in both ethnic groups (P<0.001). Bonferroni adjusted post hoc tests showed that in both ethnic groups, all sites were significantly different from each other for tibia bone total area and tibia bone total density (P<0.05), but not for tibia mass (38% site not significantly different from distal site).
Figure 4.5 Geometry outcomes by site moving up the tibial shaft length - unadjusted data
(n=17 Asian; n=48 Caucasian) SSIp=polar strength strain index, SSIx and SSiy- strength strain index with respect to x and y axes
For all three sites, tibia total density increased up the tibia shaft (distal to 38% tibia) in an almost linear fashion, and at the same rate in both groups (+276% change for Asian vs +304% change for Caucasian). Mass decreased from distal tibia to 14% tibia, and then rose again at the 38% tibia. This was the same for both ethnic groups, but the drop at the 14% tibia was more pronounced in...
Asians (-40% change in A vs -25% change in C). Tibia total bone area dropped rapidly (-66% change in A vs -55% C) from the distal tibia to the 14% tibia, whereas it dropped only slightly further (-14% change in A vs -23% C) to the 38% tibia. The rate of change for bone area was similar in Caucasians and Asians.

4.3.4 BMI relationships with tibial measurements

The associations between BMI and the tibial adaptations (tibial total density and tibial cortical thickness) seen in the Asians were examined by linear regression (Figures 4.7-4.9). The same relationships were also examined in the Caucasians for comparison. In Caucasians, there were weak, but statistically significant, positive relationships between BMI and total density at the 4% (R²=0.177, P=0.003) and 14% (R²=0.099 P=0.030) sites but not the 38% (R²=0.029 P=0.245) site. This suggests that as BMI increased total density also increased. Similar results were found for BMI and cortical thickness at the 14% (R²=0.138 P=0.009) site but not the 38% (R²=0.035 P=0.206) site. For Asians there were no significant associations between BMI and total density at the 4% (R²=0.006 P=0.761), 14% (R²=0.165 P=0.106) and 38% (R²=0.047 P=0.405) sites. There were also no significant associations between BMI and cortical thickness at the 14% (R²=0.004 P=0.810) and 38% (R²=0.091 P=0.240) sites.
Figure 4.7: Tibial total density in relation to BMI in Caucasians and Asians (n=17 Asian; n=48 Caucasian)
Figure 4.8: Tibial total density and cortical thickness in relation to BMI in Caucasians and Asians (n=17 Asian; n=48 Caucasian)
4.3.5 25(OH)D and pQCT indices

4.3.5.1 Radial measurements

Correlations between radial measurements and 25(OH)D can be seen in Table 4.7. For the radius, trabecular density (4%) was positively correlated with 25(OH)D in Asians (r=0.54 P=0.024; Figure 4.10) but not in Caucasians (r=0.06 P=0.664). No other parameters in either ethnic group were significantly associated with 25(OH)D. In Caucasians only (n=50), there were trends for a relationship between 25(OH)D and BMC (4%) (r=0.213 P=0.159); and between 25(OH)D and trabecular area (4%) (r=0.223 P=0.142), and between 25(OH)D and total area (4%) (r=0.222 P=0.142). As illustrated in Table 4.7, these trends became statistically significant when age or BMI were controlled for. Similarly, both BMC (66%) and SSIP (66%) were significantly positively correlated with vitamin D status in Caucasians (n=50) when BMI (but not age) was controlled for in the analysis (BMI adjustment: BMC r=0.379, P=0.013; SSIP r=0.298, P=0.055).
Figure 4.10: 25(OH)D concentration and radial (4%) trabecular density in Asians (n=18)
### Table 4.7: Pearson (unadjusted data) and partial (age or BMI adjusted) correlations between vitamin D and pQCT indices

<table>
<thead>
<tr>
<th>pQCT indices</th>
<th>Caucasians</th>
<th>Asians</th>
<th>n</th>
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<th>50</th>
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<td>AGE</td>
<td>BMI</td>
<td>UNADJ.</td>
<td>AGE</td>
<td>BMI</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BMC g/cm 4%</td>
<td>r</td>
<td>.213</td>
<td>.307</td>
<td>.404</td>
<td>.262</td>
<td>.329</td>
<td>.231</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>.159</td>
<td>.048</td>
<td>.008</td>
<td>.310</td>
<td>.231</td>
<td>.407</td>
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<td>r</td>
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<td>.252</td>
<td>.379</td>
<td>.136</td>
<td>.231</td>
<td>.096</td>
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<tr>
<td></td>
<td>p</td>
<td>.267</td>
<td>.108</td>
<td>.013</td>
<td>.603</td>
<td>.408</td>
<td>.733</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSIp mm^3 66%</td>
<td>r</td>
<td>.180</td>
<td>.229</td>
<td>.298</td>
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<td>.242</td>
</tr>
<tr>
<td>p .150</td>
<td>.123</td>
<td>.077</td>
</tr>
<tr>
<td>BMC 4%: BMC 38%</td>
<td>r .182</td>
<td>.182</td>
</tr>
<tr>
<td>p .244</td>
<td>.248</td>
<td>.123</td>
</tr>
<tr>
<td>CoA 14% ToA 4%</td>
<td>r -.188</td>
<td>-.189</td>
</tr>
<tr>
<td>p .227</td>
<td>.232</td>
<td>.270</td>
</tr>
</tbody>
</table>

Radius site 4% (distal) 66% (mid shaft); Tibia site 4% (distal) 14% (shaft) 38% (shaft), BMC= bone mineral content SSIp=polar strength strain index, SSIx=axial strength strain index, SSiy=axial strength strain index, ToA= total area, ToD=total density, CoA=cortical area, CoSA= cortical subarea, FLx=Fracture load with respect to x axis, Fly=Fracture load with respect to y axis, PC=periosteal circumference, EC=endosteal circumference, CT=cortical thickness, TrabA=trabecular area, TrabD=trabecular density; ≠log transformed for statistical analysis
4.3.5.2 Tibia

There was a significant positive correlation between trabecular area (4%) and 25(OH)D in Caucasians ($r=0.345$, $P=0.023$; Figure 4.11) but not in Asians ($r=0.126$, $p=0.644$). Also, there was no significant association between trabecular density (4%) and 25(OH)D in either ethnic group. For total area (4%) there was a significant positive correlation with 25(OH)D in Caucasians ($r=0.339$, $P=0.026$) but not in Asians.

![Figure 4.11: 25(OH)D concentration and tibial (4%) trabecular area in Caucasians- nb. outlier D177 removed as unfeasible value (n=47) with $R^2=0.119; p=0.023$](image)

In terms of bone strength, for Caucasians only, there was a positive relationship between 25(OH)D and SSIp (14%) ($r=0.409$, $P=0.007$; Figure 4.12), as well as between 25(OH)D and tibial total area (14%) ($r=0.340$, $P=0.026$; Figure 4.13). Similarly, for periosteal circumference (14%), in Caucasians there was a significant positive correlation with 25(OH)D ($r=0.336$, $P=0.027$; Figure 4.14). Periosteal circumference (14%), trabecular area (4%), total area (4%) and 14%) and SSIp (14%) all remained statistically significant in Caucasians, after adjustment for age and BMI. In Caucasians, the trends for a relationship between 25(OH)D and BMC (44%), trabecular area (4%) and total area (4%) became significant when BMI or age were controlled for.
Figure 4.12: 25(OH)D concentration and tibial (14%) SSIp in Caucasians (n=48)

Figure 4.13: 25(OH)D concentration and tibial (14%) total area in Caucasians (n=48)

Figure 4.14: 25(OH)D concentration and tibial (14%) Periosteal Circumference in Caucasians (n=48)

Also, the trends for BMC (66%) and SSIp (66%) became significant when BMI (but not age) was controlled for. In Asians, no parameters showed a significant relationship with 25(OH)D, for unadjusted data, or data adjusted for BMI or age. See Table 4.7 for full results of this analysis.
4.4 Discussion

The aim of the study was to compare bone geometry between South Asian and Caucasian postmenopausal women, and to assess whether there were associations between bone geometry and 25(OH)D status.

4.4.1 pQCT Radius - ethnic differences

At the distal (4%) radius, a smaller bone size and a similar BMC between Caucasians and Asians was found, with the smaller bone size in the Asians leading to increased total density. In contrast, for the mid shaft (66%) radius, although a smaller area was found, this was concurrent with less BMC, and thus similar bone density to that of Caucasians. Interestingly, cortical thickness at the 66% radius was also proportionately thicker (for overall bone size) in the Asians.

These findings suggest an ethnic difference in radial bone geometry at the distal radius, which, due to smaller bone size, are predicted to translate into poorer bone strength in Asians. Much lower radial bone strength (strength strain indexes) was predicted in Asians than Caucasians. This difference did not persist when height was controlled for, which suggests that lower bone strength in the Asians was mainly explained by their smaller bone size. As SSI calculations do not consider the thickness of the cortex, further modelling or mechanical testing would be required of the bone properties to assess whether a thicker cortex in Asian women at the diaphyseal radius increases bone strength. Also, SSI calculations were only assessed at the mid shaft radius, so this estimate did not consider the increased total density seen at the distal site. Despite the limitations of using the SSI, the existence of such poor estimated radial bone strength in Asians is a matter of concern, considering the increased risk of fracture this would predict. It is particularly of concern that slender bones may also contain more damageable bone material (Jepsen 2011).

In terms of previous research, our finding of a smaller radial bone size in Asians concurs with findings in younger South Asian women (Roy et al. 2005; Ward et al. 2007) and in other Asian groups (e.g. Chinese (Walker et al. 2011), Vietnamese (Melton et al. 2011). However, some of our findings are in contradiction to previous research. Islam et al. (2011) found that premenopausal women of Bangladeshi origin had no differences at the distal (4%) radius in total BMC, total area or trabecular density as compared to Finnish Caucasians. This supports our finding of no ethnic differences in BMC and trabecular density but contrasts with our results where a smaller bone area was found at this site in South Asians. At the mid shaft radius, Islam et al. (2011) found that the Bangladeshi women had smaller total BMC, total area, cortical area, and cortical density, but a
similar SSI to the Caucasians (Islam et al. 2011). This is again similar to our results, except that equivalent cortical density and BMC were found at this site, a thicker cortex (in proportion to bone size) and a lower SSI. Ward et al. (2007) found no differences in trabecular vBMD, total vBMD or total area at the distal radius in their premenopausal South Asian women, compared with Caucasian women (Ward et al. 2007). These results are in discordance to our older South Asian cohort who showed a smaller total area at both the distal and mid shaft radius, as compared to Caucasians, as well as increased total density at the distal radius. The most likely explanation for the differences between our study results and that of previous research is the postmenopausal status of our participants. Age and oestrogen status are important determinants of bone structure, so it is difficult to compare the results of our postmenopausal South Asian women with that of studies of premenopausal women, as all of our postmenopausal women grew up on the South Asian continent. Therefore, their childhood nutrition and lifestyle factors (e.g. exercise) are likely to be different from that of South Asian premenopausal women, who are usually born in the UK, or enter the UK at a very young age. This is likely to have affected their bone development, including that of bone morphology and peak bone mass.

4.4.2 pQCT Tibia - ethnic differences

For the distal tibia, it was found that bone in Asians is similar in structure to Caucasians, with all parts in proportion but on a smaller scale. An increased total density at this site in Asians was also found. This increased total density did not remain significant when BMI was controlled for. This suggests that the total density at this bone site was influenced by the increased weight for height in the Asians, which perhaps causes increased compression strain at this tibia area. However, the finding of no relationship between BMI and total density at the 4% site in Asians did not support this conclusion.

At the 14% tibia, as at the distal tibia, a higher total density and higher cortical density in Asians than Caucasians was found, due to increased bone mineral relative to smaller bone size. Again, it could be speculated that this may be an adaptation to offset the detrimental effect of increased body weight for height, but this was not supported by the data on relationships between BMI and tibial total density in Asians. Bone strength (SSI) in our South Asian women at the 14% site was also consistently and substantially lower (30-40%) than in the Caucasians. This was despite an increased cortical density which suggests the strength detriment was due to smaller bone size. Last, for the 38% tibia in Asians, our findings again suggested a bone that is smaller, but proportionately similar in structure to Caucasians. Also, there was an increased cortical thickness in relation to overall bone size (i.e. the same cortical thickness as that of Caucasians, but in a smaller bone) and also reduced SSI. Despite this, there was no increased bone density at this site. This is likely due to the priority
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for offsetting torsion and bending forces at the more mid shaft section of the tibia, rather than compressive strength.

In terms of previous research, there are no known South Asian data at the tibia to compare with our older South Asian sample. Tibial bone geometry has been studied in Chinese women and it is well established that Chinese women have both a thicker cortex and thicker trabeculae inside a smaller bone size than Caucasian women (Wang and Seeman 2011). These adaptations may be beneficial in improving bone strength as more bone mineral within a smaller bone size will reduce the amount of bone remodelling. This is due to a lower surface area (with the denser bone), for remodelling to take place, which is especially important during ageing (Wang and Seeman 2011). This adaptation was seen in our older South Asian women, with all sites having a smaller bone size with either equivalent mineral mass, or proportionately more mass for size than Caucasians.

Interestingly, the ethnic difference in overall bone size seen in our present study was even larger than that previously reported for older Chinese and Caucasian women by Walker et al. (2011)(Walker et al. 2011). The data reported in this chapter showed a 15-20% smaller total area at the radius and 16-38% smaller total area at the tibia in the Asians as compared with the Caucasians. This is in comparison to 10% smaller area at the distal radius and 8% smaller area at the distal tibia seen in the Chinese women (Walker et al. 2011). As described above, a higher cortical thickness (in relation to size) in Asians at the 38% tibia as compared to Caucasians was found. An increased cortical thickness has also been seen in other groups of East Asian women at the radius and tibia, and femoral neck (Walker et al. 2011; Kim et al. 2011). Walker et al. (2011)(Walker et al. 2011) found a significantly higher cortical thickness (+10% tibia, +18% distal radius) in older Chinese women compared to older Caucasian women. Conversely, the dataset presented in this chapter did not show the increased trabecular density at the distal sites that has been seen in some (Walker et al. 2011; Walker et al. 2009) but not all studies (Melton et al. 2011) of East Asian women. The differences in resolution in the two different types of pQCT and site positioning used in our study as compared with other studies may explain some of this variation, as well as the different ethnic groups studied.

4.4.3 Relationships between BMI and geometry of the tibia

As mentioned above, it would be expected that some of the tibial adaptations may be due to increased BMI in the Asian women. However, the lack of statistical significance for the relationship between tibial total density or cortical thickness and BMI in the Asians does not support this hypothesis. Nonetheless, it is possible that this analysis was underpowered, due to small numbers of Asian participants. There was a weak, but statistically significant relationship between BMI and total density, and between BMI and cortical thickness in the Caucasian women, for whom a larger sample
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existed. Also, in some cases (e.g. 14% total density), the Asians had a larger correlation coefficient than the Caucasians, although this was not statistically significant. A retrospective power calculation suggested that the power for this analysis was 71.4% in Caucasians and 50.6% in Asians. This suggests that at least for some bone parameters, the small sample size is affecting the significance of the results. Alternatively, it can be speculated that the ethnic differences seen in this study are due to adaptations to improve strength in a smaller bone. It is not known whether there are other ethnic differences in the growth or ageing processes, which could also underpin these differences. In terms of ageing, there is some research which suggests there is a very fast rate of bone loss after the menopause in South Asian women (Kadam et al. 2010).

4.4.4 Associations between the radius and 25(OH)D status

In Asians, distal radial trabecular density was associated with 25(OH)D. In Caucasians, for both age and for BMI adjusted data, at the distal radius, BMC and bone area were positively associated with 25(OH)D. Also, in this group, at the mid shaft radius, BMC and SSIp became significantly associated with 25(OH)D after BMI adjustment. Therefore, 25(OH)D appears to be positively associated with some size, mass and strength parameters in Caucasians, and with trabecular density in Asians. Increased radial trabecular density with increased 25(OH)D has not been seen in previous studies that have assessed the relationship between 25(OH)D and bone geometry. Our results are in contrast to the two other studies which have assessed pQCT bone geometry and 25(OH)D in Caucasians. The study of older women found that ultra-distal radial total bone density to be associated with 25(OH)D status (Boonen et al. 1997). Also, another study found a trend for an increased cortical BMD with increased 25(OH)D concentration in young to middle aged men (Viljakainen et al. 2009). It is unclear, why, unlike these studies, our data did not show an association between 25(OH)D and total bone density or between 25(OH)D and cortical bone density. It is likely to be due to the different bone site measured (i.e. distal and mid shaft radius, rather than ultra-distal), or the population group being studied which explains these differences (women rather than men).

Unfortunately, there are no previous pQCT data to compare our results with for the South Asian group. Still, it is of note that none of the previous studies in Caucasians have found an association between trabecular vBMD and 25(OH)D. Our finding of increased trabecular density in Asians with higher 25(OH)D concentration can be explained by the increased bone mineralization when vitamin D is sufficient, compared with deficient. This may also explain the increased mass in the Caucasians as vitamin D status improves. The mechanism for the positive relationship between vitamin D status and bone size, and between vitamin D and bone strength in Caucasians is not clear. As BMI were controlled for in the analysis, it is unlikely that differences in body weight, diet or physical activity
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by vitamin D status can explain these findings. It is possible that these findings may be effects from childhood, whereby the Caucasian women who have good vitamin D status today also had good vitamin D status as children. Adequate vitamin D in childhood may have increased the growth rate of the bone, increasing bone size. Nonetheless it is still unclear why the Caucasian women did not show increased density, as well as mass and size. It is possible that in these Caucasian women the bone prioritized increased size (i.e. by increasing periosteal circumference) rather than increasing bone density.

4.4.5 Associations between the tibia and 25(OH)D status

The increased trabecular density seen at the radius with increased vitamin D status was not also seen at the tibia in the Asians. However, as with the radius, Caucasians showed positive associations between 25(OH)D and tibial size, mass and strength parameters, although many of these relationships were only significant after adjustment for age or BMI. Other studies have found similar results. For example, increased tibial BMC and cross sectional area were found in newborn infants whose mother had increased serum 25(OH)D (Viljakainen et al. 2010). Also, in older women, Pedone et al. (2010) found that 25(OH)D was associated with tibial cortical BMD (Pedone et al. 2010). Our results support the above findings of Viljakainen et al. (2010) of increased mass and size parameters in Caucasians, albeit in a different population group (i.e. older women rather than infants). Unlike Perdone et al. (2010) our data did not show an increased cortical BMD in our older women with increased 25(OH)D concentration. It is not clear why this would be the case, as both our study, and that of Perdone et al. (2010) were undertaken in older Caucasian women, of similar age. It is also unclear why, unlike the radius, Asians did not show an increased trabecular density with increased 25(OH)D at the tibia. It might be expected that vitamin D status would affect both sites, rather than just one site. In Caucasians, the same arguments for the effects of childhood vitamin D status apply here for the tibia, as described for the radius above. The fact that many parameters were still significant after BMI adjustment suggests that exercise loading and body weight were not able to totally explain the positive relationship between 25(OH)D and bone mass, size and strength in Caucasians.

4.4.6 Limitations

There are some limitations to this work that should be considered. It is likely that there are bone architectural differences between South Asians and Caucasians which are not measurable without the use of HR-pQCT (e.g. connectivity, number and thickness of individual trabeculae). Also, the scope of our study was restricted to non-frail postmenopausal women (age range 55-75 years old). Our Asian women were of relatively high socio-economic status and reasonably good health, so are
likely to be an optimistic description of the true bone health of the wider population group. The lack of a significant relationship between 25(OH)D and some of the bone parameters in the Asians may be partly due to lower participant numbers, giving an underpowered analysis. Some of our Asian women had only recently started vitamin D supplements within the last three years, which may partly explain why there was a positive relationship between 25(OH)D and bone density, but not bone size.

4.5 Conclusion

To conclude, it was found that older South Asian women have smaller bone size, and heavier body weight for skeletal size, but have some structural adaptations to improve strength. These include increased total density at the distal radius and distal tibia, as well as a higher total density and higher cortical density at the 14% tibia. There was a proportionately thicker cortical thickness in relation to bone size at the 66% radius and 38% tibia. However, despite these adaptations, the wider implications are that South Asian women are still likely to be of higher fracture risk than same-age Caucasians, because of the substantial negative contribution to strength of a smaller bone size. A positive relationship between vitamin D status and radial trabecular density was found in Asians, as well as between vitamin D status and size, mass and strength of the radius and tibia in Caucasians. These associations may be due to differences in mineralisation or bone growth according to vitamin D status.
Part II: Muscle strength – ethnic differences and associations with vitamin D status
4.6 Introduction

There has been much interest in differences in muscle function between population sub-groups. For instance, there is a known difference in muscle function by gender, with males usually scoring higher on muscle strength than females and younger adults scoring higher than older adults (Shaunak et al. 1987). There is a particularly notable decrease in muscle strength with ageing, which has been attributed to the increased sarcopenia and fat infiltration of muscle fibres, as well as a reduction in the amount of type II fibres (Lexell 1995). As a result, it is expected that of all adults, older females have the poorest muscle function.

There has also been some investigation as to whether there are ethnic differences in muscle strength. Some studies have found ethnic differences in muscle function (Liang et al. 2007; Davis et al. 1999; Haas, Krueger, and Rohlfsen 2012; Araujo et al. 2010). Conversely, some other studies have found no ethnic differences in muscle function (Walts et al. 2008; Araujo et al. 2010; Schiller et al. 2000; Ostchega et al. 2004). The above studies examined a wide range of ages as well as different ethnic groups. Mostly, these groups were those prevalent in the US (Caucasian, Black, Hispanic, Chinese and Japanese populations). However, there is a lack of studies assessing muscle function in South Asian groups. Two exceptions are the study by Shaunak et al. (1987) which found that Caucasians had higher quadriceps strength than South Asians and another study which compared older Indian men (dwelling in India) with same-age American men, and found poorer grip strength in the Indian men (Albert et al. 2005). One limitation of the Albert et al. (2005) study is the cross country comparison, which is a major confounder in the analysis. These two studies are the only published research in the literature base comparing Caucasian and South Asian muscle function. However, there is no known literature, to the author’s knowledge which has assessed older South Asian women and muscle function.

It is known that vitamin D status is important for muscle function. Vitamin D has both genomic and non-genomic effects of muscle (reviewed by Ceglia 2008). Hence, many studies have assessed the relationship between 25(OH)D and muscle strength and function. A large number of studies show a positive relationship between 25(OH)D and better physical performance in older persons on a variety of muscle strength tests (Houston et al. 2011; Houston et al. 2009; Toffanello et al. 2012; Boersma et al. 2012; Muir and Montero-Odasso 2011; Houston et al. 2011). Despite this, little research has also assessed whether there are ethnic differences in the relationship between 25(OH)D and muscle function in different ethnic groups. One USA study assessed 25(OH)D and muscle strength in Black, White and Hispanic men, and found no association between 25(OH)D and muscle strength (Ceglia et al. 2011). A small number of studies have demonstrated a positive relationship between 25(OH)D
and muscle strength in South Asian populations (Krishnaveni et al. 2011) (Gupta et al. 2010; Goswami et al. 2012). These studies did not compare the response of the South Asians with other ethnic groups, so it was not possible to assess ethnic differences. Also, in the latter two studies, calcium was supplemented, which could also have been part of the mechanism for the muscle function improvements seen with improved vitamin D status.

The known low concentrations of 25(OH)D in South Asian women in the UK (Lowe et al. 2010; Darling et al. 2013) suggests a need for research to assess whether poorer vitamin D status is associated with worse muscle function in this population group. Also, it is known that South Asian women have a smaller lean body mass than Caucasian women even when body size is equal (Lear et al. 2009). This would suggest a potential detriment to muscle strength in South Asian women, as compared with Caucasian women.

The purpose of this analysis was two-fold. The first was to assess whether there were differences in muscle function in older South Asian women, as compared with same-age Caucasian women. The second purpose was to assess whether 25(OH)D status is associated with muscle function and physical performance in these two ethnic groups. This was achieved by assessing grip strength and time to walk measures, as well as self-reported musculoskeletal pain and difficulties with tasks of daily living. It was hypothesised that the South Asian group would have lower muscle strength and physical performance, as well as increased self-reported musculoskeletal pain than Caucasians, due to poorer vitamin D status and ethnic differences in muscle size. Also, it was hypothesised that there would be a significant positive relationship between vitamin D status and muscle function and physical performance in both ethnic groups.

4.7 Methods

4.7.1 Study design, protocol, recruitment, and biochemistry

Subject recruitment procedures were as described previously in Chapter 2 (Section 2.2.1). The study protocol was reported in Chapter 2 (Section 2.2.2). Details of biochemical analyses for 25(OH)D and PTH were given in Chapter 2, (Section 2.1.4). For this analysis, 25(OH)D had been measured using HPLC. Information is provided in chapter 2 for the scoring of the muscle and bone pain questionnaire (Section 2.3.5). This questionnaire can also be viewed in Appendix F.
### 4.7.2 Statistical analysis

The analyses included those women on vitamin D supplements and those on bone medications. Independent t-tests were used to assess group differences in grip strength and stand to walk time. Fisher Exact tests were used to analyse the association between ethnicity and individual question scores from the muscle and bone pain questionnaire, due to the nominal nature of the data. A Mann Whitney test was used to analyse group differences in the total score on the daily living scale, as this was ordinal data. Data from the stand to walk test were not normally distributed so were log transformed prior to statistical analysis.

For associations between 25(OH)D and muscle function measures, Pearson’s and partial correlations were run. An exception was for the scores on the bone pain questionnaire, which were of ordinal nature and were analysed using Spearman’s Rho. All these analyses were repeated for the association between PTH and muscle function.

Partial correlation analyses were undertaken to assess the association between 25(OH)D and muscle strength, whilst controlling for confounders. Age and BMI were controlled for as potential confounders in all analysis, as these factors are known to be associated with 25(OH)D concentration, as well as being associated with muscle function. The exceptions to this adjustment were the scores from the muscle and bone questionnaire which were not continuous data, so partial correlations were not appropriate. Also, the grip strength correlations were adjusted for age and height, instead of BMI. This is due to the fact that grip strength in the arm is positively correlated with height and arm length (Koley and Pal Kaur 2011). Consequently, height is likely to more appropriate as an adjustment than is BMI.

### 4.8 Results

#### 4.8.1 Participant characteristics

Participant characteristics for the sub-samples analysed in Sections (4.8 - 4.9) are presented here. For all analyses, (except 25(OH)D vs muscle and bone pain scores) the Caucasian women were older by around 2-3 years (p≤0.05). Also, in all analyses, the Asians had a BMI that was 3-4Kg/m² higher than the Caucasians (p≤0.05). Other parameters were not significantly different by ethnicity. Tables 4.8 to 4.13 suggest that mean and SD for height, weight, BMI, age and time from menopause were similar for all the sub analyses. This gives confidence that the small amount of missing data for
some parameters for some women in each analysis was not deviating from the general characteristics of the cohort (see Appendix AA for characteristics of the whole 2010 postmenopausal cohort n=82).

Table 4.8: Participant characteristics - sub-sample of post-menopausal women with grip strength measurements (RH data) (n=81)

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a Age ranges: Asians 58-71 years; Caucasians 59-75 years ≠measured by HPLC

Table 4.9: Participant characteristics – sub sample of post-menopausal women with ‘stand to walk’ measurements (n=79)

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<td>62.89</td>
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<td>17.67</td>
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<td>25(OH)D nmol/L≠</td>
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<td>Walking (mins per day)</td>
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a Age ranges: Asians=58-70 years; Caucasians=59-75years ≠measured by HPLC

Table 4.10: Participant characteristics- sub sample of post-menopausal women with muscle and bone pain questionnaire scores (n=78)

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a Age ranges: Asians 58-71 years; Caucasians 59-75 years ≠measured by HPLC
Table 4.11: Participant characteristics- sub-sample of postmenopausal women with both 25(OH)D and grip strength measurements (right hand) (n=74)

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<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height (cm)</strong></td>
<td>Mean 161.94</td>
<td>Mean 154.94</td>
<td>SD 6.45</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>65.84</td>
<td>69.53</td>
<td>10.75</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.18</td>
<td>28.93</td>
<td>4.42</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>65.85</td>
<td>63.47</td>
<td>4.67</td>
</tr>
<tr>
<td><strong>Time since menopause</strong></td>
<td>16.44</td>
<td>18.00</td>
<td>6.82</td>
</tr>
<tr>
<td><strong>25(OH)D nmol/L</strong></td>
<td>83.86</td>
<td>57.40</td>
<td></td>
</tr>
<tr>
<td><strong>Walking (mins per day)</strong></td>
<td>70.36</td>
<td>52.38</td>
<td>50.26</td>
</tr>
</tbody>
</table>

a Age ranges: Asians=58-71 years; Caucasians=59-75 years ≠measured by HPLC

Table 4.12: Participant characteristics- sub-sample of postmenopausal women with both 25(OH)D and stand to walk measurements (n=72)

<table>
<thead>
<tr>
<th></th>
<th>CAUCASIAN n=55</th>
<th>ASIAN n=17</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height (cm)</strong></td>
<td>Mean 161.94</td>
<td>Mean 154.96</td>
<td>SD 6.45</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>65.84</td>
<td>69.12</td>
<td>10.75</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.18</td>
<td>28.77</td>
<td>4.42</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>65.85</td>
<td>63.00</td>
<td>4.67</td>
</tr>
<tr>
<td><strong>Time since menopause</strong></td>
<td>16.44</td>
<td>18.75</td>
<td>6.82</td>
</tr>
<tr>
<td><strong>25(OH)D nmol/L</strong></td>
<td>83.98</td>
<td>55.04</td>
<td></td>
</tr>
<tr>
<td><strong>Walking (mins per day)</strong></td>
<td>70.81</td>
<td>52.38</td>
<td>49.75</td>
</tr>
</tbody>
</table>

a Age ranges: Asians=58-70 years; Caucasians=59-75 years ≠measured by HPLC

Table 4.13: Participant characteristics- sub-sample of postmenopausal women with 25(OH)D and muscle and bone pain scores (n=71)

<table>
<thead>
<tr>
<th></th>
<th>CAUCASIAN n=54</th>
<th>ASIAN n=17</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height (cm)</strong></td>
<td>Mean 161.95</td>
<td>Mean 154.65</td>
<td>SD 6.51</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>65.35</td>
<td>70.06</td>
<td>10.23</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.99</td>
<td>29.25</td>
<td>4.22</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>65.94</td>
<td>63.65</td>
<td>4.66</td>
</tr>
<tr>
<td><strong>Time since menopause</strong></td>
<td>16.61</td>
<td>19.25</td>
<td>6.86</td>
</tr>
<tr>
<td><strong>25(OH)D nmol/L</strong></td>
<td>83.42</td>
<td>54.52</td>
<td></td>
</tr>
<tr>
<td><strong>Walking (mins per day)</strong></td>
<td>73.08</td>
<td>51.87</td>
<td>51.37</td>
</tr>
</tbody>
</table>

a Age ranges: Asians 58-71 years; Caucasians 59-75 years ≠measured by HPLC

4.8.2 Grip strength

Independent t-tests showed that there was a statistically significant difference in mean grip strength by ethnicity (see Figure 4.15), with South Asians having a significantly lower grip strength in both left (t=4.360, P<0.001) and right hands (t=0.4856, P<0.001). Asians had 69-70% of the strength of their Caucasian counterparts for their left and right hands respectively.
ANCOVA showed that the increased grip strength in the left hand remained when age and BMI were controlled for in the analysis ($F=18.038$, $P<0.001$, $n=59$ Caucasian and $n=21$ Asian). The same result was found for the right hand ($F=22.391$, $P<0.001$, $n=60$ Caucasian and $n=21$ Asian).

### 4.8.3 Timed ‘stand to walk’

Independent t-tests showed that South Asian women (mean (SD); 8.07 (1.87) seconds) had a significantly longer stand to walk time than Caucasians (mean (SD); 6.87 (1.41) seconds) ($t=-3.151$; $P=0.002$; Figure 4.16).

Caucasians took 85% of the time that South Asians did to complete the task. ANCOVA showed that the increased stand to walk time in the Asian group remained when age and BMI were controlled for in the analysis ($F=14.037$, $P<0.001$, $n=61$ Caucasian and $n=19$ Asian).
4.8.4 Muscle and bone pain questionnaire

For the general questions relating to bone and muscle pain or weakness (Q23a, Q24, Q27a, Q29) the following results were found. Fisher Exact 2x2 tests showed that significantly more Asians reported having thigh muscle weakness than Caucasians (P=0.004). There was a non-statistically significant trend for Asians being more likely to report having had pain in the back of their thighs (P=0.068). Last, there was no difference by ethnicity in reported bone (P=0.268) or muscle pain (P=0.564) at any site (Table 4.14).

Table 4.14: Response rates for general questions on muscle weakness and muscle and bone pain: post-menopausal women by ethnicity.

<table>
<thead>
<tr>
<th>n/total n(%)</th>
<th>Q29 Thigh weakness</th>
<th>Q24 Thigh pain</th>
<th>Q23a Any bone pain</th>
<th>Q27a Any muscle pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Caucasians</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/49 (0%)</td>
<td>6/50 (12%)</td>
<td>22/53 (42%)</td>
<td>24/43 (56%)</td>
<td></td>
</tr>
<tr>
<td><strong>Asians</strong></td>
<td>4/18 (22%)</td>
<td>6/18 (33%)</td>
<td>10/17 (59%)</td>
<td>7/15 (47%)</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.004</td>
<td>0.068</td>
<td>0.268</td>
<td>0.564</td>
</tr>
</tbody>
</table>

*in last 3 years; ≠ Fisher Exact 2x2 Test

4.8.5 Daily living scale scores

The Mann-Whitney U test showed that there was a significant difference by ethnicity in scores on the difficulties with daily living scale. Asians were significantly higher on this scale than Caucasians, indicative of poorer self-reported daily functioning (Table 4.15). Still, the overall level of functioning in both groups was still reasonably good, with even the most severe cases only reaching a score of 12 (both ethnic groups had the same maximum). A median score of 3 in the Asians meaning they only had, for example, ‘some difficulty’ on three of the eight tasks, or ‘much difficulty’ on one of the eight tasks. The median score of 1 was very low in the Caucasians, indicative of reporting ‘some difficulty’ in only one of the 8 items.
Table 4.15: Scores from daily living scale (0= no difficulty, 32= severe difficulty) for postmenopausal women by ethnicity

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
<th>Mann Whitney U</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>57</td>
<td>1.82</td>
<td>2.64</td>
<td>1.00</td>
<td>2.50</td>
<td>327.5</td>
<td>0.008</td>
</tr>
<tr>
<td>Asian</td>
<td>19</td>
<td>4.53</td>
<td>4.35</td>
<td>3.00</td>
<td>9.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.6 Specific questions on the daily living scale

Fisher exact 2x4 tests showed that Asians had significantly more self-reported difficulty in bending down to pick up lightweight objects (like clothing) from the floor, lifting a ten-pound object from the floor, reaching for an object just above their head, standing up for 30 minutes, climbing stairs at a moderate pace and walking at a moderate pace on flat ground (P<0.05) (Table 4.16). However, there was no significant difference for putting on socks or tights on either foot and getting in and out of a car. For some tasks, ethnic differences were considerable, with only half as many Asians as Caucasians stating they had no difficulty with climbing stairs, and one third less Asians than Caucasians saying they had no difficulty walking on flat ground or reaching for an overhead object.

4.8.7 Association between scores on daily living scale, age and BMI

Spearman’s Rho was used to examine whether participants’ scores on the daily living scale were associated with their age and BMI, within ethnic groups (Table 4.17). There was a positive association between age and problem in bending down to pick up lightweight objects in Asians (r=0.457, P=0.043) as well as a positive association between BMI and problems getting out of a car in Asians (p=0.600, P=0.005). There was also a positive association between BMI and difficulty in climbing stairs in Asians (r=0.462 P=0.04). A positive relationship was found between age and difficulty in climbing stairs in Caucasians (r=0.257 P=0.05) and between age and problems standing up for 30 mins in Caucasians (r=0.299, P=0.02). Also, there was a significant association between inability to walk at a moderate pace on flat ground for both BMI (r=0.261, P=0.048) and age (r=0.430, P=0.001) in Caucasians; and in Asians for BMI only (r=0.619, P=0.004). Last, for total sum of scores on the daily living scale and age, there was a significant positive association with age for Caucasians (r=0.301, P=0.023) and BMI in Asians (r=0.474, P=0.04).
Musculoskeletal Health and Vitamin D status

Table 4.16: Individual item scores on the daily living scale (Q15-Q22) for postmenopausal women by ethnicity≠

<table>
<thead>
<tr>
<th>Group, (answer)*</th>
<th>Q15 Bending for lightweight objecta</th>
<th>Q16 10lb object from floora</th>
<th>Q17 Overhead objectab</th>
<th>Q18 Putting on socksb</th>
<th>Q19 Getting in/out of carb</th>
<th>Q20 Standing: 30 minsce</th>
<th>Q21 Climbing stairsac</th>
<th>Q22 Walking on flat groundc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian n</td>
<td>59</td>
<td>57</td>
<td>58</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>Asian n</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Caucasians (0)</td>
<td>48 (81.4%)</td>
<td>29 (50.9%)</td>
<td>55 (94.8%)</td>
<td>45 (76.3%)</td>
<td>48 (78%)</td>
<td>48 (81.4%)</td>
<td>51 (86.4%)</td>
<td>54 (91.5%)</td>
</tr>
<tr>
<td>Asians (0)</td>
<td>11 (55%)</td>
<td>9 (47.4%)</td>
<td>12 (60%)</td>
<td>12 (60%)</td>
<td>13 (65%)</td>
<td>10 (52.6%)</td>
<td>9 (45%)</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Caucasians (1)</td>
<td>11 (18.6%)</td>
<td>22 (38.6%)</td>
<td>3 (5.2%)</td>
<td>11 (18.6%)</td>
<td>13 (22%)</td>
<td>11 (18.6%)</td>
<td>8 (13.6%)</td>
<td>4 (6.8%)</td>
</tr>
<tr>
<td>Asians (1)</td>
<td>9 (45%)</td>
<td>2 (10.5%)</td>
<td>7 (35%)</td>
<td>5 (25%)</td>
<td>6 (30%)</td>
<td>5 (26.3%)</td>
<td>8 (40%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Caucasians (2)</td>
<td>0 (0%)</td>
<td>5 (8.8%)</td>
<td>0 (0%)</td>
<td>2 (3.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>Asians (2)</td>
<td>0 (0%)</td>
<td>6 (31.6%)</td>
<td>1 (5%)</td>
<td>3 (15%)</td>
<td>1 (5%)</td>
<td>4 (21.1%)</td>
<td>3 (15%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Caucasians (3)</td>
<td>-</td>
<td>1 (1.8%)</td>
<td>-</td>
<td>1 (1.7%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asians (3)</td>
<td>-</td>
<td>2 (10.5%)</td>
<td>-</td>
<td>0 (0%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fisher Exact P</td>
<td>0.035</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.185</td>
<td>0.143</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* power based activities; *flexibility based activities; * stamina based activities; ≠59 Caucasians and 20 Asians answered the muscle and bone pain questionnaire. *Answer on question 0=no difficulty; 1=some difficulty, 2=much difficulty; 3=unable to do %= per cent of women in ethnic group answering question with that answer, ¥ PB was identical for all these test results.
Table 4.17: Spearmans rho correlations between BMI and age with scores on the tasks daily living scale (Q15-Q22)

<table>
<thead>
<tr>
<th>Task of daily living question</th>
<th>CAUCASIAN</th>
<th>ASIAN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMI</td>
<td>Age</td>
</tr>
<tr>
<td>Bending down to pick up lightweight objects (like clothing) from the floor</td>
<td>0.164</td>
<td>0.205</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>Lifting a ten-pound object (like 10 bags of sugar) from the floor</td>
<td>0.161</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>0.236</td>
<td>0.265</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>Reaching for an object just above your head, such as a jar on the shelf</td>
<td>0.181</td>
<td>-0.021</td>
</tr>
<tr>
<td></td>
<td>0.177</td>
<td>0.876</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>58</td>
</tr>
<tr>
<td>Putting on socks or tights on either foot</td>
<td>0.156</td>
<td>0.243</td>
</tr>
<tr>
<td></td>
<td>0.241</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>Getting in and out of a car</td>
<td>-0.038</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>0.775</td>
<td>0.275</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>Standing up for 30 minutes</td>
<td>0.074</td>
<td><strong>0.299</strong></td>
</tr>
<tr>
<td></td>
<td>0.583</td>
<td><strong>0.022</strong></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>Climbing stairs at a moderate pace</td>
<td>0.182</td>
<td><strong>0.257</strong></td>
</tr>
<tr>
<td></td>
<td>0.171</td>
<td><strong>0.050</strong></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>Walking at a moderate pace on flat ground</td>
<td><strong>0.261</strong></td>
<td>0.430</td>
</tr>
<tr>
<td></td>
<td><strong>0.048</strong></td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>Sum of daily living scale</td>
<td>0.155</td>
<td><strong>0.301</strong></td>
</tr>
<tr>
<td></td>
<td>0.254</td>
<td><strong>0.023</strong></td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>57</td>
</tr>
</tbody>
</table>

n.b. bold typeface highlights statistically significant results r=spearmans rho correlation coefficient

### 4.8.8 Back pain

The frequency of back pain was ascertained to assess whether this was a likely explanation for any of these ethnic differences in the daily living scale. A Fisher exact 2x2 test showed that there was no significant ethnic difference in self-reported back pain in the last six months (A=65% yes, C=63.3% yes, p=0.558; n=20 A and n=60 C).
**4.8.9 Relationships between Vitamin D status and Grip Strength**

Correlations between grip strength and 25(OH)D were weak (r=0.17-0.22; Table 4.18) with no statistically significant Pearson’s or partial correlations between 25(OH)D and grip strength in either ethnic group, for unadjusted, age adjusted or height adjusted data.

**Table 4.18: Pearson’s and partial correlations between mean grip strength and 25(OH)D and PTH**

<table>
<thead>
<tr>
<th>Hand</th>
<th>25(OH)D</th>
<th>Unadj.</th>
<th>Age</th>
<th>Height</th>
<th>PTH*±</th>
<th>Unadj</th>
<th>Age</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td>r</td>
<td>0.226</td>
<td>0.217</td>
<td>0.208</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.097</td>
<td>0.115</td>
<td>0.132</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>55</td>
<td>55</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r</td>
<td>0.042</td>
<td>0.092</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.866</td>
<td>0.717</td>
<td>0.784</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Left</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td>r</td>
<td>0.264</td>
<td>0.257</td>
<td>0.249</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.053</td>
<td>0.064</td>
<td>0.072</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r</td>
<td>0.334</td>
<td>0.372</td>
<td>0.371</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.163</td>
<td>0.129</td>
<td>0.130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Unadj.=Unadjusted data ±log transformed data

The exception was for unadjusted data for the relationship between 25(OH)D and left hand grip strength in Caucasians (r=0.264, P=0.053, n=54). This relationship showed only a trend when age (P=0.064) or height (P=0.072) were controlled for in the analysis. There was no significant association between log PTH and grip strength for either hand, in either ethnic group.

**4.8.10 Relationship between vitamin D status and stand to walk time**

Data for the relationship between stand to walk time and 25(OH)D and the equivalent data for PTH are illustrated in Table 4.19. For unadjusted data, the only statistically significant association was between 25(OH)D and stand to walk time in Caucasians (r=-0.269, P=0.045). The negative correlation suggests that in Caucasians, as vitamin D increased, stand to walk time decreased. For age adjusted data, this negative relationship between 25(OH)D and walk time still remained in Caucasians. The relationship was not still significant when BMI was controlled for in the analysis.
Table 4.19 Pearson’s and partial correlations between mean stand to walk time and 25(OH)D and PTH

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D</th>
<th></th>
<th>PTH*±</th>
<th></th>
<th>25(OH)D</th>
<th></th>
<th>PTH*±</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadj.</td>
<td>Age</td>
<td>Height</td>
<td>Unadj.</td>
<td>Age</td>
<td>Height</td>
<td></td>
</tr>
<tr>
<td>25(OH)D</td>
<td></td>
<td></td>
<td></td>
<td>Caucasians</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>r</td>
<td>0.269</td>
<td>-0.271</td>
<td>-0.233</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p</td>
<td>0.045</td>
<td>0.046</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>56</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>Cauc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.317</td>
<td>0.332</td>
<td>0.305</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.017</td>
<td>0.013</td>
<td>0.023</td>
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<td>56</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.354</td>
<td>0.372</td>
<td>0.353</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>As.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.535</td>
<td>0.200</td>
<td>0.537</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.164</td>
<td>0.156</td>
<td>0.181</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
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<td></td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

*Unadj. = Unadjusted data, ±log transformed data

For PTH, there was a significant positive relationship with stand to walk time in Caucasians (r=0.317, P=0.017, n=56), which remained significant when controlling for age (P=0.013) or height (P=0.023). However, there was no significant relationship between PTH and stand to walk time in the Asian group, although the r coefficient values were of a similar size to that of the Caucasians.

4.8.11 Relationship between Vitamin D status and muscle or bone pain

First, the total score for the daily living questionnaire was correlated with 25(OH)D and with PTH using Spearman’s Rho correlations. There were no significant associations between 25(OH)D or PTH with daily function in either of the ethnic groups (see Table 4.20). Next, in order to assess the association between level of difficulty reported for each of the daily living separate tasks and vitamin D status, Spearman’s rho correlations were also run for these variables (Tables 4.21 - 4.28).

No task scores were significantly associated with 25(OH)D, although there was a trend for a positive relationship between 25(OH)D concentration and the difficulty score for putting on socks/tights in Caucasians (r=0.239 P=0.081). There was also a trend for a negative relationship between 25(OH)D concentration and difficulty in standing for 30 mins in Asians (r=-0.438 P=0.079). The only significant association between PTH and the individual questions on the daily living scale were for standing for 30 mins (Q20) in Asians (r=0.521, P=0.032, n=17), with increased PTH being associated with increased score on this scale, and thus reduced ability to perform this task.
Table 4.20: Spearman’s correlations* between Vitamin D status or PTH and Daily living scale scores for postmenopausal women by ethnicity

<table>
<thead>
<tr>
<th>25(OH)D</th>
<th>Unadj.</th>
<th>PTH±</th>
<th>Unadj.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>r 0.083</td>
<td>Caucasians</td>
<td>r -0.024</td>
</tr>
<tr>
<td>p 0.558</td>
<td>p 0.865</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n 52</td>
<td>n 52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r 0.001</td>
<td>Asians</td>
<td>r 0.163</td>
</tr>
<tr>
<td>p 0.998</td>
<td>p 0.532</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n 17</td>
<td>n 17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Unadj.=Unadjusted data ; ±log transformed data

Table 4.21: Spearman’s correlations* between Vitamin D status/PTH and Q15 Bending for lightweight object on floor

<table>
<thead>
<tr>
<th>25(OH)D</th>
<th>Unadj.</th>
<th>PTH</th>
<th>Unadj.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>r 0.046</td>
<td>Caucasians</td>
<td>r 0.074</td>
</tr>
<tr>
<td>p 0.742</td>
<td>p 0.597</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n 54</td>
<td>n 54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r 0.237</td>
<td>Asians</td>
<td>r 0.119</td>
</tr>
<tr>
<td>p 0.343</td>
<td>p 0.638</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n 18</td>
<td>n 18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Unadj.=Unadjusted data ; ±log transformed data

Table 4.22: Spearman’s correlations* between Vitamin D status/PTH and Q16 Lifting 10lb object from floor

<table>
<thead>
<tr>
<th>25(OH)D</th>
<th>Unadj.</th>
<th>PTH</th>
<th>Unadj.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>r 0.124</td>
<td>Caucasians</td>
<td>r -0.025</td>
</tr>
<tr>
<td>p 0.380</td>
<td>p 0.858</td>
<td></td>
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</tr>
<tr>
<td>n 52</td>
<td>n 52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r 0.029</td>
<td>Asians</td>
<td>r 0.122</td>
</tr>
<tr>
<td>p 0.911</td>
<td>p 0.640</td>
<td></td>
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<tr>
<td>n 17</td>
<td>n 17</td>
<td></td>
<td></td>
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</tbody>
</table>

*Unadj.=Unadjusted data ; ±log transformed data
Table 4.23: Spearman’s correlations* between Vitamin D status/PTH and Q17 Reaching for overhead object

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D</th>
<th>Unadj.</th>
<th>PTH</th>
<th>Unadj.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td></td>
<td>-0.101</td>
<td>0.150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.470</td>
<td>0.285</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>53</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r</td>
<td>0.270</td>
<td>-0.128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.278</td>
<td>0.614</td>
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<tr>
<td></td>
<td>n</td>
<td>18</td>
<td>18</td>
<td></td>
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</tbody>
</table>

*Unadj.=Unadjusted data ; ±log transformed data

Table 4.24: Spearman’s correlations* between Vitamin D status/PTH and Q18 Putting on socks

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D</th>
<th>Unadj.</th>
<th>PTH</th>
<th>Unadj.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td></td>
<td>0.239</td>
<td>0.073</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.081</td>
<td>0.602</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>54</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r</td>
<td>0.132</td>
<td>-0.136</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.602</td>
<td>0.591</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>18</td>
<td>18</td>
<td></td>
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</table>

*Unadj.=Unadjusted data ; ±log transformed data

Table 4.25: Spearman’s correlations* between Vitamin D status/PTH and Q19 Getting in/out of car

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D</th>
<th>Unadj.</th>
<th>PTH</th>
<th>Unadj.</th>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Caucasians</td>
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<td>0.058</td>
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</tr>
<tr>
<td></td>
<td>p</td>
<td>0.742</td>
<td>0.675</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>54</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r</td>
<td>0.228</td>
<td>-0.287</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.363</td>
<td>0.248</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>18</td>
<td>18</td>
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</tr>
</tbody>
</table>

*Unadj.=Unadjusted data ; ±log transformed data

Table 4.26: Spearman’s correlations* between Vitamin D status/PTH and Q20 Standing for 30 mins

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D</th>
<th>Unadj.</th>
<th>PTH</th>
<th>Unadj.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td></td>
<td>-0.034</td>
<td>-0.098</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.809</td>
<td>0.481</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>54</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r</td>
<td>-0.438</td>
<td>0.521</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.079</td>
<td>0.032</td>
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</tr>
<tr>
<td></td>
<td>n</td>
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</tbody>
</table>

*Unadj.=Unadjusted data ; ±log transformed data
Table 4.27: Spearman's correlations* between Vitamin D status/PTH and Q21 Climbing stairs

<table>
<thead>
<tr>
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<th>PTH</th>
<th>Unadj.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>r</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
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</table>

*Unadj.=Unadjusted data ; ±log transformed data

Table 4.28: Spearman's correlations* between Vitamin D status/PTH and Q22 Walking on flat ground

<table>
<thead>
<tr>
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<th>PTH</th>
<th>Unadj.</th>
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</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>r</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>r</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
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</tbody>
</table>

*Unadj.=Unadjusted data ; ±log transformed data

### 4.9 Discussion

The aim of this analysis was to assess whether there are ethnic differences in muscle function, and whether there is an association between muscle function and 25(OH)D status.

#### 4.9.1 Ethnic differences in muscle function

There was a trend for the Asians to report more thigh pain, but not more bone pain. However, they did report significantly more muscle weakness in their thighs than Caucasians. This is known to be one of the clear signs of vitamin D deficiency. In terms of the tasks of daily living scale, there were statistically significant, but not biologically relevant differences in scores, with Asians scoring slightly higher on overall reported difficulty. When looking at individual tasks, the Asians reported significantly higher scores on reaching for an object just above their head, standing up for 30 minutes, climbing stairs at a moderate pace and walking at a moderate pace on flat ground, suggestive of increased difficulty with these kinds of tasks. This is hard to understand considering the daily living scores overall showed little ethnic difference. It might be that some tasks are more
Musculoskeletal Health and Vitamin D status

problematic, but not others. Particularly, it appears that Asian women reported themselves worse at tasks requiring some form of stamina (e.g. walking, climbing) than those tasks requiring flexibility (e.g. putting on shoes) or power (e.g. lifting a heavy object). These self-reports concur with the slighter longer stand to walk time observed in the Asian women than in Caucasian women. Ethnic differences in back pain were excluded as a potential explanation for ethnic differences in self-reported task scores. Alternatively, it may be that ethnicity is not the main influence on scores on these tasks. The within ethnic group correlations for both BMI and age were reasonably large, and statistically significant in many cases. This suggests that BMI and age may be exerting some influence on the ratings of task difficulty. This seems especially likely considering it was the stamina based activities which the Asian women reported faring worse with. More research is required to investigate this further.

Few previous studies have assessed differences between South Asian and Caucasian women on measures of difficulty in tasks of daily living. One review of ethnic inequalities in health in the UK reported prevalence rates for specific activities that are limited by health related problems (Davey Smith et al. 2000). This review used data from the Fourth National Survey of Ethnic Minorities 1993-1994 (Nazroo 1997) and found that after standardisation for gender and age, all South Asians had a prevalence rate of 16% for difficulty climbing several flights of stairs, and 8% for difficulty climbing one set of stairs. This was in comparison to 12% and 4% respectively for the White Caucasians (Nazroo 1997). Similarly, all South Asians had a prevalence rate of 12% for problems with carrying groceries (vs. 8 per 100 for White Caucasians), a prevalence of 5% for problems with bathing/dressing (vs. 3% for White Caucasians) (Nazroo 1997). Interestingly, ability to bend, kneel or stoop was similar between all South Asians and White Caucasians, but for most tasks South Asians fared worse than White Caucasians (Nazroo 1997). Our results support the findings of the above survey, in that South Asians showed slightly more difficulty on most (but not all) tasks, but the difference as compared with White Caucasians was not always large.

Asians had weaker grip strength than Caucasians, but there was no significant difference in the stand to walk test. The weaker grip strength could be explained by smaller muscle and bone size in the Asians than in the Caucasians. This difference was not solely due to differences in height or age, as the difference was still apparent when these parameters were controlled for in the statistical analysis. The equivalent stand to walk test between the two groups was surprising, considering the known increased prevalence of musculoskeletal pain in South Asian women (Allison et al. 2002). This difference persisted even when age and BMI were controlled for in the analysis. As with tasks of daily living, there are no previous South Asian data to compare these findings with for grip strength and time to walk test. Our work supports that of Haas et al. (2012) who found significant ethnic differences in grip strength and contradicts the work of Araujo et al. (2010) who found did not find
an ethnic difference in grip strength (Araujo et al. 2010). The Araujo et al. (2010) study assessed 30-70 year old Black, Caucasian and Hispanic men, which could explain the differences found as compared with our study of postmenopausal South Asian and Caucasian women. It is likely that the results of these studies vary due to the heterogeneous nature of the study populations in terms of ethnic groups being compared, and different gender and age groups.

No previous work has specifically assessed South Asian populations for the stand to walk test. However, one study found ethnic differences in walking speed and ability to stand from a chair (Davis et al. 1999). Also, another study found ethnic differences in leg muscle strength (Liang et al. 2007). Our research contradicts these studies. One possible reason is the fact that the Davis et al. (1999) compared Japanese persons with Caucasians, and the Liang et al. (2007) study compared Chinese women with Hispanic and Caucasian women. Thus, it could be that East Asian populations have lower muscle strength than Caucasian populations. Schiller et al. (2000) and Ostchega et al. (2004) found no ethnic difference in knee strength (Schiller et al. 2000; Ostchega et al. 2004). The Schiller et al. (2000) study compared Hispanic women with Caucasian women, whilst the Ostchega et al. (2004) study compared Hispanic, Black and Caucasians. Although, the results of our analysis did not specifically measure knee strength, they support the findings of these two studies, albeit using a different test of lower extremity strength as well as a different ethnic group. Further tests on both upper and lower body strength are required to assess muscle strength and function in older South Asian women further.

The lack of ethnic difference found could also be due to the fact that this group of Asian women were not severely deficient in vitamin D. Indeed, 63% of Asians (vs. 36% of Caucasians) were on vitamin D supplements and only 5% of Asians were severely deficient enough (25nmol/L) to be likely to show vitamin D deficiency induced mobility problems. Consequently, they did not show a large difference in vitamin D status, as compared with Caucasians.

### 4.9.2 Associations between muscle function and vitamin D status

It was found that scores on the daily living questionnaire were not associated with vitamin D status, or PTH in either ethnic group. There are no previous data assessing the relationship between tasks of daily living and 25(OH)D in South Asians. However, the few studies done in elderly Caucasians show an increased ability to do daily tasks with increasing 25(OH)D (Skalska et al. 2012; Houston et al. 2011). It was surprising that no overall scores on the daily living questionnaire were associated with vitamin D status. Also, none of the individual component tasks of the daily living scale were significantly associated with vitamin D status. One task in Caucasians (positive relationship; putting on socks/tights) and one task in Asians (negative relationship; standing for 30 minutes) showed a
trend for a relationship with 25(OH)D concentration. The Caucasian result was a positive association, which would be the opposite of what would be expected. It is unclear why these two tasks showed an association with 25(OH)D, when the others did not, as these did not use different muscle groups, or grossly different function to some of the other tasks (e.g. standing is similar to walking). The only significant association between PTH and scores of daily living was for standing for 30 mins, whereby increased PTH was associated with poorer self-related ability to perform this task in Asians only. There was no association between PTH and the overall daily living score in either ethnic group.

It could be that the tools used in this analysis (e.g. questionnaire, nominal Fisher exact test analysis) were not sensitive enough, or didn’t have enough statistical power, to detect functional differences that may vary with vitamin D status. As discussed above, the finding of a reasonably large, significant association between BMI and age with difficulty in the tasks of daily living is relevant here. The magnitude of these within ethnic group associations were larger than that for 25(OH)D and task difficulty, suggesting BMI and age may be a more important influence on difficulty with these tasks than is vitamin D status. Similarly, in one published study, all associations between 25(OH)D and functional performance disappeared when BMI was controlled for in the analysis (McDermott et al. 2012).

There was no association between 25(OH)D and grip strength in either ethnic group, with or without adjustment for age and height. This finding contradicts that of Toffanello et al. (2012) who found increased grip strength with increased 25(OH)D concentration. This result can be explained by the fact that the above results were seen in men only and not seen in women (Toffanello et al. 2012). This would explain why it was not seen in either our South Asian or Caucasian women, in that it may be a gender specific relationship. The relationship may also be age specific. The increased grip strength seen with increased 25(OH)D in the Krishnaveni et al. (2011) study, was seen in South Asian children, not adults (Krishnaveni et al. 2011). It may simply be that grips strength is not related strongly to 25(OH)D status. This would explain why young Indian women did not have improved grip strength when supplemented with vitamin D in the study by Goswami et al. (2012) (Goswami et al. 2012).

In Caucasians stand to walk time was significantly associated with vitamin D status and PTH. As vitamin D decreased, (or PTH increased), time to walk increased, as expected. This supports the finding by Toffanello et al. (2012) that increased 25(OH)D was associated with increased walking speed in male Caucasians; and improved ability to do chair stands in female Caucasians (Toffanello et al. 2012). Also, this work supports that of Muir et al. (2011) who found increased 25(OH)D status
to be associated with results on the stand to walk test (Muir and Montero-Odasso 2011). Thus, our results for the Caucasian group support that of previous research.

In Asians, there were some trends for a negative association between 25(OH)D and stand to walk time of a similar magnitude to that of the Caucasians, but these results did not reach statistical significance. A retrospective statistical analysis with the results from the analysis (Caucasian mean (SD)= 6.87 (1.41) n 61; vs. Asian mean (SD)= 8.07 (1.87) n 19) gives a statistical power of 73.2%, which is only slightly below the desirable statistical power of 80%. At a power of 73.2%, the lack of statistical significance is likely to be not due to the smaller sample size in the Asian group. To be sure of this, at 80% power, n 28 Asian and n 28 Caucasian subjects would be required. There are no previous South Asian data on walking speed to compare these results with.

One of the clear limitations to this work is the problem of self-reporting muscle and bone pain, including type of pain and location. As with the results for bone geometry and vitamin D status, the high usage of vitamin D supplements in the Asian group may be affecting the results for the relationship between vitamin D and muscle function. Again, small subject numbers in the Asian group may be causing some analyses to be underpowered. This is especially likely considering the similar magnitude of the correlation coefficients see in this analysis.

### 4.10 Conclusion

Asians had a grip muscle strength that was weaker than Caucasians, but showed no difference in function as assessed by the stand to walk test. Vitamin D status was not associated with grip strength in either ethnic group. Also, overall daily living score was not associated with vitamin D status in either ethnic group, neither were the component tasks assessed within this questionnaire. Of all the muscle function tests, stand to walk was the only measure associated with vitamin D status, and only in Caucasians. Therefore, vitamin D status may only be associated with some aspects of bone geometry, and with some aspects of muscle function. The lack of significant results throughout this chapter, in the Asian group, despite some strong trends, is likely be due to lack of statistical power due to small sample sizes. Further research with a larger sample of Asians is required to resolve this issue. To summarise, the picture is complex, with Asians having poorer musculoskeletal health than Caucasians on some aspects (e.g. bone geometry, grip strength), and not others (muscle function whilst walking, tasks of daily living). To complicate matters, vitamin D status is associated with musculoskeletal health with some (e.g. stand to walk) but not all of these aspects.
CHAPTER 5- Light exposure, sleep and vitamin D
5.1 Introduction

There has been some investigation into ethnic differences in rest-activity cycles and sleep quality, with potential ethnic differences in the characteristics of sleep found in both adults (Stepnowsky et al. 2003; Sanford et al. 2006; Durrence and Lichstein 2006; Profant et al. 2002; Song et al. 2011; Hall et al. 2009) and children (Olds et al. 2010; Gradisar et al. 2011). Of interest, in adults, Jean-Louis et al (2000) found a significant difference in total sleep time and sleep efficiency by gender and ethnic group, with men from ethnic minority groups (Hispanic, African American or Asian) having the poorest sleep (Jean-Louis et al. 2000). Whilst there may be socio-economic or genetic reasons for ethnic differences in sleep quality, differences in light exposure may also play a role in explaining these differences. Kripke et al (2004) found that Caucasian women had a higher light exposure than Black or Hispanic women and that longer, but poorer quality sleep was associated with reduced light exposure (Kripke et al. 2004). Unfortunately, there has been no subsequent research into ethnic differences in light exposure.

In addition to the lack of studies of differences in light by ethnicity, there is a lack of data assessing sleep quality in a wide range of different ethnic groups. Most studies assessing ethnicity and sleep quality have been conducted in the USA and have focussed on African American, Caucasian, Hispanic, and Japanese populations. Few studies have looked at South Asian populations, and there is a lack of key data concerning sleep patterns in this ethnic group. To date there are no known studies assessing the prevalence of sleep related problems in migrant South Asian groups. Only a small amount of research has assessed sleep problems in South Asians residing in South Asia. Furthermore, most of this research in South Asians has been confined to studies of men, with sleep problems commonly being attributed to sleep apnoea, caused by obesity (Agrawal et al. 2011). One recent study that did assess both genders found that 10% of South Asian women (compared with 8% of men) reported insomnia (Panda et al. 2012). However, there has been no research to assess whether insomnia rates, or sleep quality differs between Caucasian and South Asian women. Lower UVB exposure has been found in UK dwelling South Asian than Caucasian women (Darling et al. 2013). This study suggests that it is likely that the South Asian women’s total light exposure is lower than that of Caucasian women. If South Asian women have lower light exposure and poorer sleep than Caucasian women, this would be of public health concern. This is due to the detrimental effects of poor sleep on general health, including increased risk of chronic diseases such as cardiovascular disease, diabetes and obesity (Shankar, Syamala, and Kalidindi 2010).

It is unknown whether the poorer vitamin D status seen in South Asian women living in the UK (Darling et al. 2013) would lead to any problems with sleep quality. Moreover, it is unknown
whether vitamin D is associated with sleep quality. Recently, vitamin D has been associated with a wide variety of mental and behavioural conditions of the central nervous system (Lansdowne and Provost 1998; Anglin et al. 2013; Eyles et al. 2013). However, there has been very little research assessing whether sleep is associated with vitamin D. Recent work has mapped the human brain to identify areas containing the vitamin D receptor (VDR) and vitamin D hydroxylase enzyme (Eyles et al. 2005). A particularly high concentration of the VDR was found in the hypothalamus, especially in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) (Eyles et al. 2005). The PVN is involved in the transmission of impulses from the SCN to the pineal gland, and VDR concentrated areas of the brain may be involved in, or interact with these circadian pathways.

However, despite all the interest in vitamin D function in the brain, and associations with mental illness, little research has specifically examined whether there is any link between vitamin D and circadian rhythms, or between vitamin D and sleep. One conference paper (Pande et al. 2009) presented data on an association between vitamin D status and sleep duration in the USA. However, this study used N-HANES survey data and thus it was not possible to adequately control for light exposure, which could be a confounding factor in this analysis. Additionally, a very recent US study found that a sample of sleep disordered patients with reportable musculoskeletal pain had a higher prevalence of vitamin D deficiency compared with published data for patients with general musculoskeletal pain, or a reference ‘healthy’ population (McCarty et al. 2013). This is an interesting result that warrants further investigation, despite the clear methodological problems of using published data in the comparison groups, rather than actual subject data collected at the time of the study. There is a clear need for further research assessing the potential link between vitamin D status and sleep quality.

In the work presented in this chapter, rest-activity cycles and individual light exposure, as well as subjectively reported sleep quality, were assessed in UK dwelling South Asian and Caucasian women. Drawing on the evidence presented above, it was predicted that South Asian women would have lower light exposure and poorer sleep quality than Caucasian women and that vitamin D status would be positively associated with light exposure and sleep quality.

5.2 Methods

5.2.1 Study design and participant characteristics

All participants who had taken part in the main study (2010) (n=123) were invited to take part in the additional light and sleep sub-study. A total of n=47 women participated in the light and sleep sub-
study (n=20 South Asian and n=27 Caucasian women). The age range of these participants was 39-75 years old, including both premenopausal and postmenopausal women. Recruitment details and study protocol are as reported in chapter 2 (Sections 2.2.1-2.2.2). Details of the participant characteristics for the whole D-FINES summer 2010 cohort are shown in Appendix AA (postmenopausal women) and Appendix AB (premenopausal women).

5.2.2 Procedure

Participants (n=47) were asked to wear a wrist worn Actiwatch for assessment of rest-activity cycles for 14 consecutive days, 24 hours a day. A subset of participants also wore an actiwatch on a neck cord during the waking hours, over the same 14 days as the wrist worn watch, in order to assess their light exposure. In total, n=35 participants (n=15 Asian and n=20 Caucasian) wore both neck and wrist Actiwatches, and n=12 (n=5 Asian and n=7 Caucasian) wore wrist worn Actiwatches only. All participants were asked to keep a record of when the watch and/or neck monitors, as appropriate, were worn and removed, as well as to keep a sleep diary (see Appendix Q for details of these records). All subjects in the sleep and light sub-study also completed the Munich Chronotype Questionnaire (MCTQ) to assess mid sleep point (MSFsc) and social jetlag (SJL)6 (see Appendix R for details of MCTQ). Additionally, all D-FINES participants in the 2010 cohort (n=110), (including those not in the light and sleep sub-study) had completed the Pittsburgh sleep quality index (PSQI; see Appendix I) during their visit to the University in the previous month.

5.2.3 Statistical analysis

5.2.3.1 Light and sleep analyses- general aspects

Details about the data cleaning and analysis processes for the light data were presented previously in chapter 2 (Section 2.2.3.2). The light analysis consisted of calculating the amount of time spent by participants over different lux thresholds (≥100 lux; ≥200 lux; ≥500 lux; ≥1000 lux and ≥2000 lux). Daily light time profiles were also produced, using the median lux at each hour of the day for the study participants.

6 MSFsc= mid sleep on free days corrected for sleep loss on workdays (MSFsc = MSF – (SDf – SDweek)/2 whereby MSF= Mid sleep on free days; SDf= Standard deviation of free days; SDweek= Standard deviation of weekdays). Social jetlag (SJL)= the difference between mid sleep times on free days and on workdays (MSF-MSW) (Roenneberg et al. 2012).
The sleep diaries were used to assess subjectively reported sleep duration, time ‘try to sleep’ and wake time. Time ‘try to sleep’ is the time that the participant reported trying to get to sleep (i.e. lights off and lying down). Wake time was the time that the participant woke up in the morning (i.e. when they first opened their eyes, not necessarily when they got out of bed). Subjectively reported sleep duration was calculated as the participant time ‘try to sleep’ minus the wake time. For example, if a participant’s time ‘try to sleep’ was 23:00 h and the wake time was 06:00 h then the subjectively reported duration was 7 h. These data were analysed as stand-alone (i.e analysed as subjectively reported sleep parameters in their own right) and also used to input information about participant bedtime and wake time to enable running of the sleep analysis software (Cambridge Neurotechnology Limited, Version 7.1)

The sleep analysis produced parametric data, including estimates of sleep latency, sleep efficiency, sleep fragmentation and sleep duration (see chapter 2, Section 2.3.4 for full descriptions of these parameters). Details about the cleaning processes for the sleep analysis are presented in chapter 2 (Section 2.2.3.3). Details of actual the sleep data analysis procedure are shown in chapter 2 (Section 2.2.3.4). In addition, assessment of the Actiwatch data was also made using the non-parametric circadian rhythm analysis (NPCRA) macro (supplied by Professor Eus van Someren). This enabled estimation of the L5, L5 onset, M10, M10 onset, amplitude, relative amplitude, IS and IV (see Chapter 2, Section 2.2.3.5 for details of the NPCRA analysis). Table 5.1 lists the NPCRA parameters produced by this analysis. Last, the PSQI was used to assess subjective sleep quality. The PSQI was scored according to standard instructions (Buysse 1989). Full details of the PSQI scoring algorithm can be found in Appendix X.

5.2.3.2 Exclusion of women with insufficient valid data

All women with less than 7 days of Actiwatch data were excluded from the sleep analysis (n=5 Asian). For the light analysis, n=6 Asian women were excluded due to malfunction of the neck monitor (n=4), or due to less than 7 days of data (n=2). All women who had been excluded from the sleep and/or light analysis, but had valid PSQI scores were retained in the PSQI analyses only. For the PSQI analysis of the whole cohort (n=112) and for the PSQI analysis of the light and sleep sub-study participants (n=47), n=4 Asian women were excluded. The reasons for this were non-completion (n=1) or incomplete PSQI completion (n=3). This led to a slight reduction in sample sizes to n=108 for the whole cohort analysis, and n=43 for the light and sleep sub-study participant analysis. Details of excluded participants from each of the analyses in this chapter can be seen in Appendix AC.
Table 5.1: NPCRA sleep parameters (Van Someren et al. 1999)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>M10 onset</td>
<td>The time when the most active 10 hours (M10) starts</td>
</tr>
<tr>
<td>M10</td>
<td>Amount of activity in the most active ten hours</td>
</tr>
<tr>
<td>L5 onset</td>
<td>The time when the least active 5 hours starts (L5)</td>
</tr>
<tr>
<td>L5</td>
<td>Amount of activity in the least active 5 hours</td>
</tr>
<tr>
<td>Amplitude</td>
<td>Amplitude=(M10 - L5)</td>
</tr>
<tr>
<td>Relative Activity</td>
<td>Relative Activity= amplitude/(M10+L10)</td>
</tr>
<tr>
<td>Relative Amplitude</td>
<td>Relative Amplitude=((M10 - L5)/(M10+L5)). The relative change in activity from M10 to L5, not absolute change</td>
</tr>
<tr>
<td>Inter-daily stability (IS)</td>
<td>The inter-daily stability (IS) is the stability of the circadian rhythm. A lower IS means a higher variation in values between days, thus a less stable rhythm</td>
</tr>
<tr>
<td>Intra-daily variability (IV)</td>
<td>Intra-daily variability (IV) is the degree of sleep and activity fragmentation, within a day. This value is increased by night-time awakening and daytime naps.</td>
</tr>
</tbody>
</table>

5.2.3.3 Statistical procedures

The four groups were assigned abbreviations for the purpose of clarity. For sections 5.3.1-5.3.5, subject numbers were too small to run inferential statistics on the two premenopausal groups, so data for these two groups are presented in a descriptive format only, for informational purposes. Data for the two postmenopausal groups only were analysed using Fisher exact tests (for nominal and ordinal data) and Independent t-tests to ascertain differences in postmenopausal group characteristics as appropriate. For the alcohol intake data, data were pooled into two categories (0-4 units per week and 5+ units per week) to enable the Fishers exact 2x2 test. Independent t-tests were also used to assess group differences in light exposure, actiwatch and sleep diary parameters. For assessing differences in PSQI score by ethnic-group, the Mann-Whitney test was instead of an Independent t-test, due to the ordinal nature of the PSQI scores.

7 POST CAUC, PRE CAUC, POST ASIAN AND PRE ASIAN. Statistically significant group differences are denoted in Tables and Figures throughout this Chapter by like superscripts.
Pearson’s correlations were used to assess the relationship between the Actiwatch parameters, light exposure and subjective derived sleep measures (time try to sleep, wake time, sleep duration from the sleep diaries as well as the PSQI) with vitamin D status. Partial correlations were used to assess whether these relationships were statistically significant when musculoskeletal pain and light exposure were controlled for in the analysis. Spearman’s rho correlations were used to test the association between PSQI scores and vitamin D status. For the matched pairs analysis, paired t-tests were used for assessing variables by ethnicity. The exception was for the PSQI data, whereby Wilcoxon matched-pairs signed rank tests were used. Standard regression models were run to assess the predictive ability of ethnicity and age (for continuous variables only). All statistical procedures were conducted using SPSS v19 (Chicago, IL) and Graph Pad v6.0 (San Diego, CA).

5.3 Results I- all participants

5.3.1 Participant characteristics: all participants

Table 5.2 presents background data for the women who took part in the light and sleep sub-study (n=47). For the postmenopausal women only, independent t-tests showed that there was a small, but statistically significant difference in age (P=0.003), as well as a significant difference in BMI (P=0.001) (Table 5.2). The POST ASIAN group had a significantly higher BMI than POST CAUC. There were no significant ethnic differences in IMD, caffeine intake (tea or coffee), mid-sleep point, or social jetlag (P>0.05). Fisher exact tests showed that there were no significant differences in use of sleep medications or in smoking status between the four groups. However, 18% (n=2) of the postmenopausal Asians reported using sleep medications, compared with 0% in the postmenopausal Caucasian group. In addition, there was a significant group difference for alcohol intake (P=0.005), with 100% of Asians consuming 4 or less units of alcohol per week compared with only 55% of Caucasians.

5.3.2 Light exposure analysis: participant characteristics

Participant characteristics for the subjects who wore a neck worn actiwatch are presented in Table 5.3 (n=35). Independent t-tests showed a significant difference between the two postmenopausal groups in age (P=0.007) and BMI (P=0.001), but not for IMD, caffeine intake (tea or coffee), mid-sleep point and social jetlag (P>0.05). Fisher exact tests showed no significant difference in use of sleep medications or smoking status. However, there was a borderline statistically significantly increased alcohol intake in the postmenopausal Caucasian group, as compared with the postmenopausal Asian group (P=0.053)
Table 5.2: Participant Characteristics - all subjects who took part in the light and sleep sub-study (n=47)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>POST CAUC</th>
<th>PRE CAUC</th>
<th>POST ASIAN</th>
<th>PRE ASIAN</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>22</td>
<td>66.4</td>
<td>4.9</td>
<td>5</td>
<td>41.8</td>
</tr>
<tr>
<td>IMD</td>
<td>21</td>
<td>13.8</td>
<td>7.7</td>
<td>5</td>
<td>14.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22</td>
<td>24.9</td>
<td>4.9</td>
<td>5</td>
<td>23.7</td>
</tr>
<tr>
<td>Caffeine- cups of tea/d</td>
<td>22</td>
<td>2.7</td>
<td>2.1</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Caffeine- cups of coffee/d</td>
<td>22</td>
<td>1.3</td>
<td>2.3</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>Mid-sleep (MSFsc)</td>
<td>19</td>
<td>3.4</td>
<td>0.5</td>
<td>5</td>
<td>4.1</td>
</tr>
<tr>
<td>Social Jetlag (SJL)</td>
<td>19</td>
<td>0.20</td>
<td>0.3</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>Sleep medication user</td>
<td>22</td>
<td>0</td>
<td>100</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Smoker</td>
<td>22</td>
<td>9</td>
<td>91±</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>0-4 units alcohol p/wk</td>
<td>54% (n=12)</td>
<td>60% (n=3)</td>
<td>100% (n=12)</td>
<td>100% (n=7)</td>
<td>*0.006</td>
</tr>
<tr>
<td>5+ units alcohol p/wk</td>
<td>46% (n=10)</td>
<td>40% (n=2)</td>
<td>0% (n=0)</td>
<td>0% (n=0)</td>
<td></td>
</tr>
</tbody>
</table>

*Independent t-test- between two postmenopausal groups only; IMD= Index Multiple Deprivation, MSFsc=Mid sleep point, SJL= Social jetlag;; *Fisher Exact 2x2 test
Out of the original n=35 participants (n=15 Asian and n=20 Caucasian) who wore a neck worn actiwatch, n=6 had AWLs that malfunctioned (n=5 Asian, n=1 Caucasian), so did not record light data. In addition, n=2 (both Asian) had less than 7 days of valid data once the data had been edited. This left n=29 participants (n=9 Asian and n=20 Caucasian) with valid data for the light analysis. The participant characteristics of this remaining n=29 are shown in Table 5.4. The characteristics of this sample were similar to that of the whole subset of participants who took part in the sleep and light sub-study, so will not be described in detail again here. However, of note there was now no statistically significant difference in age (P=0.124).

5.3.3 Light exposure analysis

5.3.3.1 Time over lux thresholds (n=29)

The mean amount of time each ethnic-menopausal status group spent daily over each lux threshold (>100 lux, >200 lux, >500 lux, >1000 lux and >2000 lux) are illustrated in Figure 5.1. The results for each individual (n=29) had been adjusted according to the accuracy of the watch they had worn (Chapter 2, Section 2.2.3.2 for details). The mean (SD) for number of valid days finally included in the analysis was 13.2 (1.1) in POST CAUC, 13.3 (1.0) in PRE CAUC, 11.3 (2.7) in POST ASIAN and 10.0 (2.0) in PRE ASIAN. An independent t-test showed a significant difference between the POST CAUC and POST ASIAN (P≤0.05) for time spent over 100 lux (P=0.015), over 200 lux (P=0.009), over 500 lux (P=0.012) and over 1000 lux (P=0.048).
Table 5.3: Participant Characteristics - all subjects who wore a neck worn actiwatch-L (n=35)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>POST CAUC</th>
<th></th>
<th></th>
<th>POST ASIAN</th>
<th></th>
<th></th>
<th>PRE ASIAN</th>
<th></th>
<th></th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>16</td>
<td>66.3</td>
<td>4.6</td>
<td>4</td>
<td>42.0</td>
<td>2.2</td>
<td>11</td>
<td>61.8</td>
<td>2.6</td>
<td>0.007</td>
</tr>
<tr>
<td>IMD</td>
<td>16</td>
<td>13.9</td>
<td>8.8</td>
<td>4</td>
<td>12.9</td>
<td>5.5</td>
<td>11</td>
<td>12.2</td>
<td>12.3</td>
<td>0.673</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16</td>
<td>24.9</td>
<td>5.4</td>
<td>4</td>
<td>23.3</td>
<td>2.3</td>
<td>11</td>
<td>32.0</td>
<td>4.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Caffeine- cups of tea/d</td>
<td>16</td>
<td>3.0</td>
<td>2.3</td>
<td>4</td>
<td>1.8</td>
<td>2.1</td>
<td>11</td>
<td>3.0</td>
<td>1.1</td>
<td>0.985</td>
</tr>
<tr>
<td>Caffeine- cups of coffee/d</td>
<td>16</td>
<td>1.3</td>
<td>2.6</td>
<td>4</td>
<td>1.5</td>
<td>1.3</td>
<td>10</td>
<td>0.1</td>
<td>0.3</td>
<td>0.102</td>
</tr>
<tr>
<td>Mid-sleep (MSFsc)</td>
<td>14</td>
<td>3.5</td>
<td>0.5</td>
<td>4</td>
<td>3.8</td>
<td>0.6</td>
<td>9</td>
<td>3.7</td>
<td>1.0</td>
<td>0.172</td>
</tr>
<tr>
<td>Social Jetlag (SJL)</td>
<td>14</td>
<td>0.2a</td>
<td>0.3</td>
<td>4</td>
<td>1.0a</td>
<td>0.3</td>
<td>9</td>
<td>0.4</td>
<td>0.6</td>
<td>0.375</td>
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</table>

<table>
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<tr>
<th>Parameter</th>
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<th>% Yes</th>
<th>% No</th>
<th>n</th>
<th>% Yes</th>
<th>% No</th>
<th>n</th>
<th>% Yes</th>
<th>% No</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep medication user</td>
<td>16±</td>
<td>0</td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>100</td>
<td>9</td>
<td>22</td>
<td>78</td>
<td>0.120</td>
</tr>
<tr>
<td>Smoker</td>
<td>16±</td>
<td>0</td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>100</td>
<td>11</td>
<td>0</td>
<td>100</td>
<td>#</td>
</tr>
</tbody>
</table>

- 0-4 units alcohol p/wk: 63% n=10, 50% n=2, 100% n=10, 100% n=4
- 5+ units alcohol p/wk: 37% n=6, 50% n=2, 0% n=0, 0% n=0

*Independent t-test- between two postmenopausal groups only; IMD= Index Multiple Deprivation, MSFsc=Mid sleep point, SJL= Social jetlag; *Fisher Exact 2x2 test ± includes n=1 smoker #cannot calculate Fisher’s exact as all cells constant;
### Table 5.4: Participant Characteristics - all subjects with valid light data (≥7 days) from neck worn actiwatch-L (n=29)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>POST CAUC</th>
<th>PRE CAUC</th>
<th>POST ASIAN</th>
<th>PRE ASIAN</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>16</td>
<td>66.3</td>
<td>4.6</td>
<td>4</td>
<td>42.0</td>
</tr>
<tr>
<td>IMD</td>
<td>16</td>
<td>13.9</td>
<td>8.8</td>
<td>4</td>
<td>12.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16</td>
<td>24.9</td>
<td>5.4</td>
<td>4</td>
<td>23.3</td>
</tr>
<tr>
<td>Caffeine- cups of tea/d</td>
<td>16</td>
<td>3.0</td>
<td>2.3</td>
<td>4</td>
<td>1.8</td>
</tr>
<tr>
<td>Caffeine- cups of coffee/d</td>
<td>16</td>
<td>1.3</td>
<td>2.6</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>Mid-sleep (MSFsc)</td>
<td>14</td>
<td>3.5</td>
<td>0.5</td>
<td>4</td>
<td>3.8</td>
</tr>
<tr>
<td>Social Jetlag (SJL)</td>
<td>14</td>
<td>0.2</td>
<td>0.3</td>
<td>4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% Yes</th>
<th>% No</th>
<th>n</th>
<th>% Yes</th>
<th>% No</th>
<th>n</th>
<th>% Yes</th>
<th>% No</th>
<th>n</th>
<th>% Yes</th>
<th>% No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep medication user</td>
<td>16</td>
<td>0</td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>100</td>
<td>3</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Smoker</td>
<td>16±</td>
<td>0</td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>100</td>
<td>6</td>
<td>0</td>
<td>100</td>
<td>3</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0-4 units alcohol p/wk</td>
<td>63% (n=10)</td>
<td>50% (n=2)</td>
<td>100% (n=5)</td>
<td>100% (n=3)</td>
<td>0.262</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5+ units alcohol p/wk</td>
<td>37% (n=6)</td>
<td>50% (n=2)</td>
<td>0% (n=0)</td>
<td>0% (n=0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Independent t-test- between two postmenopausal groups only; IMD= Index Multiple Deprivation, MSFsc=Mid sleep point, SJL= Social jetlag; ≠ Fisher Exact 2x2 test ± includes n=1 smoker ≠ cannot calculate Fisher’s exact as all cells constant.
Figure 5.1: Time spent per day in light levels over 100, 200, 500, 1000 and 2000 lux by ethnic-menopausal group. Independent t-test results refer to the two postmenopausal groups. Premenopausal data are shown for reference purposes only.

However, there was no statistically significant difference for time spent over 2000 lux (P=0.135).

5.3.3.2 Daily light time profiles (n=29)

Daily time profiles for the four ethnic menopausal groups are shown in Figure 5.2. In terms of timings of the main light exposure periods, these were similar for the two Asian groups, falling between 09:30 h and 18:30 h. This was similar to the postmenopausal Caucasian group, although this group had an earlier start for the light exposure period (07:30 h) than the two Asian groups. The premenopausal Caucasian light exposure was spread even further over the whole day, from 06:30 h to 19:30 h. It was also different from the other three groups in that it had multiple light bursts.
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(07:30 h, 11:30 h, 14:30 h and 16:30 h). There was large variability between the premenopausal Caucasians at the 07:30 h and 14:30 h time points. This came from the fact that on average, one woman at the 07:30 h time point had a moderate amount of light exposure (around 600 lux), and another woman had a high light exposure at 14:30 h (around 2000 lux). Peaks for the other three ethnic-menopausal status groups were at 16:30 h (postmenopausal Caucasian), 13:30 h (postmenopausal Asian) and 17:30 h (premenopausal Asian). In terms of overall intensity of light received, there was a trend for the two Caucasian groups to have a higher mean median lux throughout the day than the two Asian groups, with the premenopausal Asian group receiving the least light per day. Total area under the curve for the four groups for the 24 hour period was: Postmenopausal Caucasian (4950 units); Premenopausal Caucasian (5121 units); Postmenopausal Asian (3057 units) and Premenopausal Asian (1810 units).
Figure 5.2 Daily light time profiles for the ethnic-menopausal status groups
5.3.4 Actigraphic sleep parameters: subject characteristics

All actiwatch parameters were normally distributed according to the Kolmogorov-Smirnov test, except for sleep latency and mean wake bout time. The distributions of these two variables were successfully normalised using log transformation.

Subject characteristics for participants with complete Actiwatch (wrist worn) data for ≥7 days (n=42), split by ethnic-menopausal group, can be seen in Table 5.5. Independent t-tests showed that there were no significant group differences in IMD or for caffeine intake (from tea). There was a significant ethnic difference in caffeine intake from coffee (P=0.022), although this was only a small difference at around 1 cup per day higher in the POST CAUC group. As seen previously, there was a significant difference in age (P=0.006) and BMI (P<0.001). There were also no significant differences between the ethnic-menopausal status groups for mid sleep time (MSFsc) (P=0.256) and social jetlag (SJL) (P=0.286). Fisher exact tests showed no significant group difference in the usage of sleep medications, despite a trend for 25% of the postmenopausal Asian group, vs. 0% of the other three groups reporting usage. There was no difference in smoking status by group. However, there was a significant difference for alcohol intake (P=0.03) with 100% of the POST ASIAN group consuming less than 4 units per week, compared with only 54% of the POST CAUC group.

A summary of all results produced from the Actiwatch analysis, by ethnic-menopausal status group is shown in Appendix AD. The following sections illustrate in more detail the results for sleep latency (Section 5.3.4.1), sleep efficiency (Section 5.3.4.2) sleep fragmentation (Section 5.3.4.3) and sleep duration (Section 5.3.4.4). Individual subject data for the actigraphic sleep parameters (sleep latency, sleep efficiency, sleep fragmentation and sleep duration) over the two week period are illustrated in Appendix AE.
Table 5.5: Participant Characteristics- all subjects with complete Actiwatch (wrist worn) data for >7 days (n=42)

| Parameter                  | POST CAUC | PRE CAUC | POST ASIAN | PRE ASIAN | P<  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>22</td>
<td>66.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.9</td>
<td>5</td>
<td>41.8&lt;sup&gt;ce&lt;/sup&gt;</td>
</tr>
<tr>
<td>IMD</td>
<td>21</td>
<td>13.8</td>
<td>7.7</td>
<td>5</td>
<td>14.9</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>22</td>
<td>24.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.9</td>
<td>5</td>
<td>23.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caffeine- cups of tea/d</td>
<td>22</td>
<td>2.7</td>
<td>2.1</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Caffeine- cups of coffee</td>
<td>22</td>
<td>1.3</td>
<td>2.3</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>Mid-sleep (MSFsc)</td>
<td>19</td>
<td>3.4</td>
<td>0.5</td>
<td>5</td>
<td>4.1</td>
</tr>
<tr>
<td>Social Jetlag (SJL)</td>
<td>19</td>
<td>0.2</td>
<td>0.3</td>
<td>5</td>
<td>1.3</td>
</tr>
</tbody>
</table>

| Sleep medication user     | n         | % Yes    | % No  | n         | % Yes    | % No  | n         | % Yes    | % No  | n         | % Yes    | % No  | n         | % Yes    | % No  | 0.064  |
| Smoker                    | 22        | 0        | 100   | 5         | 0        | 100   | 8         | 25       | 75    | 4         | 0        | 100   | 5         | 0        | 100   | >0.999 |

| 0-4 units alcohol p/wk    | 54% (n=12) | 60% (n=3) | 100% (n=9) | 100% (n=5) | 0.030 |
| 5+ units alcohol p/wk     | 46% (n=10) | 40% (n=2) | 0% (n=0)   | 0% (n=0)   |  

<sup>1</sup>Independent t-test- between two postmenopausal groups only; IMD= Index Multiple Deprivation, MSFsc=Mid sleep point, SJL= Social jetlag; *Fisher Exact 2x2 test ± includes n=1 smoker
5.3.4.1 Sleep latency

Individual and mean (±SD) sleep latency is shown in Figure 5.3. An Independent samples t-test showed no significant difference by ethnic-menopausal status group in sleep latency ($t=0.362$, $P=0.720$).

Figure 5.3: Individual and mean (±SD) sleep latency in each ethnic-menopausal status group. Independent samples t-test result refers to the two postmenopausal groups only.

5.3.4.2 Sleep efficiency

Individual and mean sleep efficiency is shown in Figure 5.4.

Figure 5.4: Individual and mean (±SD) sleep efficiency (%) in each ethnic-menopausal status group. Independent samples t-test result refers to the two postmenopausal groups only.

One-way ANOVA showed a significant ethnic difference in sleep efficiency ($t=4.023$, $P<0.001$), with POST CAUC having a higher sleep efficiency than POST ASIAN by 7.5%.
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5.3.4.3 Sleep fragmentation

Individual and mean sleep fragmentation is shown in Figure 5.5.

Figure 5.5: Individual and mean (±SD) fragmentation index in each ethnic-menopausal status group. Independent samples t-test result refers to the two postmenopausal groups only.

An Independent t-test showed a significant ethnic difference in sleep fragmentation (F=4.154, P<0.001), with POST CAUC having a lower sleep fragmentation than POST ASIAN by 12%.

5.3.4.4 Sleep duration

Individual and mean sleep duration is shown in Figure 5.6. An Independent t-test showed a significant difference in actual sleep time (t=2.718, P=0.011) between POST CAUC and POST ASIAN, with POST CAUC sleeping longer by 0.8 Dec (h) (i.e. 48 minutes)

Figure 5.6: Individual and mean (±SD) actual sleep time in each ethnic-menopausal status group. Independent samples t-test result refers to the two postmenopausal groups only.
5.3.4.5 Non Parametric Circadian Rhythm Analysis (NPCRA)

Summary details of the NPCRA parameters of each ethnic-menopausal status group can be seen in Table 5.6 and Figure 5.7. Independent t-tests showed a significant difference in amplitude (P=0.037) and relative amplitude (P=0.003), but not in IS (P=0.101) or IV (P=0.322).

Independent t-tests showed a significant ethnic menopausal status group difference in L5 (P=0.024) with a non-statistically significant trend for a difference in M10 (P=0.092). There was no significant ethnic difference in L5 onset (P=0.921) or M10 onset (P=0.437) (Figure 5.8).
Table 5.6: Summary data for NPCRA parameters n=42

<table>
<thead>
<tr>
<th>Parameter</th>
<th>POST CAUC</th>
<th></th>
<th></th>
<th>PRE CAUC</th>
<th></th>
<th></th>
<th>POST ASIAN</th>
<th></th>
<th></th>
<th>PRE ASIAN</th>
<th></th>
<th></th>
<th>*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>P</td>
</tr>
<tr>
<td>IS</td>
<td>22</td>
<td>0.6</td>
<td>0.1</td>
<td>5</td>
<td>0.6</td>
<td>0.1</td>
<td>10</td>
<td>0.5</td>
<td>0.1</td>
<td>5</td>
<td>0.5</td>
<td>0.1</td>
<td>0.101</td>
</tr>
<tr>
<td>IV</td>
<td>22</td>
<td>0.8</td>
<td>0.1</td>
<td>5</td>
<td>0.7</td>
<td>0.1</td>
<td>10</td>
<td>0.9</td>
<td>0.2</td>
<td>5</td>
<td>0.8</td>
<td>0.2</td>
<td>0.322</td>
</tr>
<tr>
<td>L5</td>
<td>22</td>
<td>10.5</td>
<td>4.4</td>
<td>5</td>
<td>21.3</td>
<td>8.5</td>
<td>10</td>
<td>22.6</td>
<td>14.0</td>
<td>5</td>
<td>19.5</td>
<td>10.1</td>
<td>0.024</td>
</tr>
<tr>
<td>L5 onset (Dec.h)</td>
<td>22</td>
<td>24.8</td>
<td>1.1</td>
<td>5</td>
<td>25.3</td>
<td>1.1</td>
<td>10</td>
<td>24.8</td>
<td>1.0</td>
<td>5</td>
<td>24.7</td>
<td>1.00</td>
<td>0.921</td>
</tr>
<tr>
<td>M10</td>
<td>22</td>
<td>333.2</td>
<td>85.2</td>
<td>5</td>
<td>440.7</td>
<td>56.1</td>
<td>10</td>
<td>275.4</td>
<td>91.6</td>
<td>5</td>
<td>320.0</td>
<td>46.8</td>
<td>0.092</td>
</tr>
<tr>
<td>M10 onset (Dec.h)</td>
<td>22</td>
<td>8.7</td>
<td>0.7</td>
<td>5</td>
<td>8.7</td>
<td>1.2</td>
<td>10</td>
<td>9.1</td>
<td>1.5</td>
<td>5</td>
<td>9.7</td>
<td>1.2</td>
<td>0.437</td>
</tr>
<tr>
<td>Amplitude</td>
<td>22</td>
<td>322.7</td>
<td>83.4</td>
<td>5</td>
<td>419.5</td>
<td>53.5</td>
<td>10</td>
<td>252.8</td>
<td>84.4</td>
<td>5</td>
<td>300.4</td>
<td>40.0</td>
<td>0.037</td>
</tr>
<tr>
<td>Relative Amplitude</td>
<td>22</td>
<td>0.9</td>
<td>0.02</td>
<td>5</td>
<td>0.9</td>
<td>0.03</td>
<td>10</td>
<td>0.9</td>
<td>0.07</td>
<td>5</td>
<td>0.9</td>
<td>0.04</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Independent t-test between two postmenopausal groups only; IS = inter-daily stability, IV = intra-daily variability, L5 = 5 hours with lowest activity, L5onset = time of onset of 5 hours of lowest activity, M10 = 10 hours with most activity, M10onset = time of onset of 10 hours of most activity, Amplitude = (M10 - L5), Relative amplitude = Amplitude/(M10+L10).
Figure 5.7: Individual and mean (±SD) for inter-daily stability, IS (A); inter-daily variability, IV (B); amplitude (C) and relative amplitude (D) by ethnic menopausal status group. *Independent t-test- between two postmenopausal groups only.
Figure 5.8: Individual and mean (±SD) for L5 (A); L5 onset (B); M10 (C) and M10 onset (D) by ethnic menopausal status group. *Independent t-test between two postmenopausal groups only

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5.3.5 Subjective sleep parameters

The parameters in Section 5.3.5 were drawn from the sleep diaries or PSQI questionnaires completed by the participants and are subjective (self-reported) in nature. The complete results for the subjective sleep parameters for the ethnic-menopausal status groups drawn from the sleep diary (n=42) as well as the PSQI data for the whole cohort (n=108) and for the participants enrolled in the light and sleep sub-study (n=43) are shown in Appendix AF. This section will focus on results for sleep duration, time ‘try to sleep’, wake time and global PSQI scores. Subjectively reported sleep duration, time ‘try to sleep’ and wake time were taken from the participant sleep diaries. These parameters and how they were derived were previously reported in Section 5.2.3.

5.3.5.1 Sleep duration

An independent t-test showed no significant difference by ethnic-menopausal status group in subjectively reported sleep duration (F=0.755, P=0.456; Figure 5.9).

![Figure 5.9: Individual and mean (±SD) for subjective sleep duration in each ethnic-menopausal status group. Independent samples t-test result refers to the two postmenopausal groups only.]

5.3.5.2 Time ‘try to sleep’

An Independent t-test showed no significant difference by ethnic-menopausal status group in time ‘try to sleep’ (t= -0.985, P=0.398; Figure 5.10).
5.3.5.3 Wake time

An Independent t-test showed no significant difference by group in wake time ($t=0.118$, $P=0.908$; Figure 5.11).

5.3.5.4 PSQI scores: Whole summer 2010 cohort (n=108)

The following data (Sections 5.3.5.4 - 5.3.5.5) came from the PSQI questionnaire. From Table 5.7, it can be seen that for premenopausal women, 33% of Caucasians and 74% of Asians met the criteria for no sleep disorder$^8$ (score $\leq 5$). For the postmenopausal women, the equivalent results were 58% of Caucasians and 41% of Asians. Therefore, 17% less postmenopausal Asian women than postmenopausal Caucasian women and 41% less premenopausal Asian than premenopausal Caucasians women met the criteria for no sleep disorder (Table 5.7). The Kruskall-Wallis test also showed a trend for a difference between the groups in PSQI score, but this did not reach statistical significance ($P=0.055$; Figure 5.12).

---

$^8$ A PSQI score $>5$ has been defined as indicative of a sleep disorder (Buysse 1989).
Table 5.7: n and % scoring as no sleep disorder (PSQI≤5)

<table>
<thead>
<tr>
<th>Group</th>
<th>n (% scoring ≤5 on PSQI (no sleep disorder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POST CAUC n=60</td>
<td>35 (58%)</td>
</tr>
<tr>
<td>PRE CAUC n=19</td>
<td>14 (74%)</td>
</tr>
<tr>
<td>POST ASIAN n=17</td>
<td>7 (41%)</td>
</tr>
<tr>
<td>PRE ASIAN n=12</td>
<td>4 (33%)</td>
</tr>
</tbody>
</table>

Figure 5.12: Global PSQI score - whole summer 2010 cohort

For the whole cohort, none of the PSQI sub-scales (n=7) showed significant ethnic-menopausal status group differences apart from sleep duration (DURAT) (P=0.042) and sleep medication (MEDS) (P=0.001). For the sleep duration (DURAT) sub-scale, POST ASIAN had significantly higher scores than POST CAUC (P<0.001) and PRE CAUC (P<0.001) but not for PRE ASIAN (P=0.326). For sleep medications (MEDS), POST ASIAN was also higher than POST CAUC (P=0.001) and PRE CAUC (P=0.005), but not PRE ASIAN (P=0.058). These subgroup analyses all suggest an ethnic difference, but not a menopausal status difference, for DURAT (sleep duration) and MEDS (sleep medication use).

5.3.5.5 PSQI scores: Sleep and light sub-study participants only (n=43)

PSQI data are presented here for those who completed the sleep and light-sub study (n=43; nb. n=4 of the light and sleep study participants incorrectly completed the PSQI; or had a non-returned PSQI questionnaire). For the postmenopausal women, 50% of Asians and 73% of Caucasians met the PSQI criteria for no sleep disorder. However, for premenopausal women, 100% of Caucasians, but only 25% of Asians met the criteria for no sleep disorder (Table 5.8). Hence, it seems that the sleep in the two groups of Asian women was generally reported as poor.
Table 5.8: n and % scoring as no sleep disorder (PSQI≤5) - n=43

<table>
<thead>
<tr>
<th>Group</th>
<th>n (%) scoring ≤5 on PSQI (no sleep disorder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POST CAUC n=22</td>
<td>16 (73%)</td>
</tr>
<tr>
<td>PRE CAUC n=5</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>POST ASIAN n=12</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>PRE ASIAN n=4</td>
<td>1 (25%)</td>
</tr>
</tbody>
</table>

In addition, the Mann Whitney test (postmenopausal groups only due to small sample size in premenopausal groups) showed that there was a significant difference in PSQI score (P=0.039; Figure 5.13).

Figure 5.13: Individual and mean (± SD) for global PSQI score- sleep and light sub-study participants only. Mann-Whitney test refers to postmenopausal group differences.

For participants enrolled in the light and sleep sub-study (n=43), similar results were found to that of the whole cohort (n=108). The only sub-scales showing statistically significant differences between the ethnic-menopausal status groups were DURAT (P=0.003) and MEDS (P=0.005). For DURAT, POST ASIAN was higher in score than POST CAUC. For MEDS, POST ASIAN was significantly higher than POST CAUC.

5.3.6 Regression analysis

5.3.6.1 Sleep fragmentation

When the ethnicity parameter was run in a regression model, 29.8% of sleep fragmentation was explained (model 1, Table 5.9). When age and BMI were also added to the model (model 2), ethnicity was the strongest predictor of sleep fragmentation index (Beta=0.448, P=0.011), with Asians having a 0.448 SD higher sleep fragmentation index than Caucasians (Table 5.9). Neither age (Beta=-0.149, P=0.304) nor BMI (Beta=0.133, P=0.431) had no significant, independent ability to predict sleep fragmentation. Explained variance only increased from 29.8% (P<0.001) in model 1 to 32.2% in model 2 (P=0.002), showing age and BMI had only contributed an extra 2.4% to explaining
sleep fragmentation. Overall, using the estimates of the second model, 32.2% of sleep fragmentation index was explained by ethnicity, age and BMI.

Table 5.9: Regression models for sleep fragmentation index (n=42)

<table>
<thead>
<tr>
<th>Model 1 Parameter</th>
<th>Unstandardised coefficients</th>
<th>Standardised coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>15.191</td>
<td>3.538</td>
</tr>
<tr>
<td>Ethnicity≠</td>
<td>10.133</td>
<td>2.458</td>
</tr>
</tbody>
</table>

ANOVA F =16.994 , P < 0.001

Explained variance R² = 0.298 (adjusted R² = 0.281 )

<table>
<thead>
<tr>
<th>Model 2 Parameter</th>
<th>Unstandardised coefficients</th>
<th>Standardised coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>19.705</td>
<td>8.916</td>
</tr>
<tr>
<td>Ethnicity≠</td>
<td>8.316</td>
<td>3.106</td>
</tr>
<tr>
<td>Age</td>
<td>-0.139</td>
<td>0.134</td>
</tr>
<tr>
<td>BMI</td>
<td>0.217</td>
<td>0.272</td>
</tr>
</tbody>
</table>

ANOVA F = 6.013 , P =0.002

Explained variance R² = 0.322 (adjusted R² = 0.268 )

*Dependent variable= ≠ dummy variable (Caucasian=0 and Asian=1

5.3.6.2 Sleep efficiency

When the ethnicity parameter was entered in a regression model, ethnicity was a significant predictor of sleep efficiency (P<0.001; model 1), explaining 36.1% of the variance (Table 5.10). When age and BMI were also added to the model (model 2), ethnicity was the strongest predictor (Beta=-0.540, P=0.001), with Asians having a 0.54 SD reduced sleep efficiency than Caucasians. Age had a smaller, but significant, independent ability to predict sleep efficiency (Beta=0.296, P=0.028). BMI showed no significant, independent ability to predict sleep efficiency (Beta= -0.015, P=0.919). Explained variance by the model increased from 36.1% (P<0.001) in model 1 (ethnicity only) to 44.4% (P<0.001) in model 2 (ethnicity and age). Overall, model 2 showed that 44.4% of sleep efficiency was explained by ethnicity, age and BMI.

5.3.6.3 Other sleep parameters

Regression models, using both ethnicity, BMI and age as predictors, were not statistically significant for actual sleep time (F=1.800, P=0.164, R²=0.055; n=42) subjective sleep duration (F=0.902, P=0.449, R²=0.066; n=42), time ‘try to sleep’ (F=0.421, P=0.739, R²=0.032; n=42), wake time (F=0.498, P=0.686, R²=0.038; n=42) or log sleep latency (F=0.972, P=0.416, R²=0.071; n=42).
Table 5.10: Regression model for sleep efficiency (n=42)

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Parameter</th>
<th>Unstandardised coefficients</th>
<th>Standardised coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>SE</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>95.003</td>
<td>2.410</td>
</tr>
<tr>
<td>Ethnicity≠</td>
<td></td>
<td>-7.959</td>
<td>1.674</td>
</tr>
</tbody>
</table>

ANOVA F = 22.601, P < 0.001

Explained variance $R^2$ = 0.361 (adjusted $R^2$ = 0.345 )

<table>
<thead>
<tr>
<th>Model 2</th>
<th>Parameter</th>
<th>Unstandardised coefficients</th>
<th>Standardised coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>SE</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>83.198</td>
<td>5.763</td>
</tr>
<tr>
<td>Ethnicity≠</td>
<td></td>
<td>-7.159</td>
<td>2.008</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>0.198</td>
<td>0.086</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>-0.018</td>
<td>0.176</td>
</tr>
</tbody>
</table>

ANOVA F = 10.114, P < 0.001

Explained variance $R^2$ = 0.444 (adjusted $R^2$ = 0.400 )

*Dependent variable= ≠ dummy variable (Caucasian=0 and Asian=1)

5.3.6 Association between vitamin D and actigraphic sleep parameters

Pearson’s correlations were run between actigraphic (AWL) parameters and vitamin D status (Table 5.11). In Caucasians, there were no significant associations between vitamin D status and any of the actigraphic parameters. In Asians, there was a significant negative association between 25(OH)D concentration and log sleep latency ($r$ = -0.562, $P$ = 0.036, n=14). This suggests, in Asians, that higher vitamin D status is associated with shorter actigraphic sleep latency.

Partial correlations were also run to assess whether adjusting for the confounder of musculoskeletal pain influenced the above results. Correlations were run for bone and muscle pain separately. When controlling for bone pain (n=23 Caucasian, n=11 Asian), all relationships between actigraphic parameters and vitamin D status were still not statistically significant, except for a significant positive relationship between vitamin D status and log sleep latency in Caucasians ($r$ = 0.426, $p$ = 0.048, n=23). When adjusting for muscle pain (n=21 Caucasian, n=8 Asian), there were no significant associations between vitamin D status and any of the sleep parameters in Caucasians or in Asians ($P$ > 0.05). Last, there were also no significant relationships between vitamin D status and sleep parameters when adjusting for outdoor light exposure (time spent over 1000 lux) (n=20 Caucasian, n=8 Asian).
Table 5.11: Summary of correlation between AWL parameters and vitamin D status - all participants
(n=41) ±

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucans</th>
<th></th>
<th></th>
<th>Asains</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>n</td>
<td>r</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>Sleep duration- actual</td>
<td>-0.036</td>
<td>0.857</td>
<td>27</td>
<td>0.101</td>
<td>0.732</td>
<td>14</td>
</tr>
<tr>
<td>Actual wake time</td>
<td>-0.117</td>
<td>0.561</td>
<td>27</td>
<td>0.028</td>
<td>0.924</td>
<td>14</td>
</tr>
<tr>
<td>Sleep latency±</td>
<td>0.361</td>
<td>0.065</td>
<td>27</td>
<td>-0.562</td>
<td>0.036</td>
<td>14</td>
</tr>
<tr>
<td>Mean sleep bout time</td>
<td>0.000</td>
<td>&gt;0.999</td>
<td>27</td>
<td>0.183</td>
<td>0.532</td>
<td>14</td>
</tr>
<tr>
<td>Mean wake bout time±</td>
<td>-0.005</td>
<td>0.980</td>
<td>27</td>
<td>0.338</td>
<td>0.238</td>
<td>14</td>
</tr>
<tr>
<td>Actual sleep %</td>
<td>0.100</td>
<td>0.620</td>
<td>27</td>
<td>0.053</td>
<td>0.858</td>
<td>14</td>
</tr>
<tr>
<td>Actual wake %</td>
<td>-0.100</td>
<td>0.620</td>
<td>27</td>
<td>-0.053</td>
<td>0.858</td>
<td>14</td>
</tr>
<tr>
<td>Sleep efficiency %</td>
<td>-0.012</td>
<td>0.951</td>
<td>27</td>
<td>0.223</td>
<td>0.444</td>
<td>14</td>
</tr>
<tr>
<td>Sleep bouts</td>
<td>-0.070</td>
<td>0.729</td>
<td>27</td>
<td>-0.336</td>
<td>0.241</td>
<td>14</td>
</tr>
<tr>
<td>Wake bouts</td>
<td>-0.075</td>
<td>0.708</td>
<td>27</td>
<td>-0.333</td>
<td>0.244</td>
<td>14</td>
</tr>
<tr>
<td>Immobile (mins)</td>
<td>-0.134</td>
<td>0.505</td>
<td>27</td>
<td>0.057</td>
<td>0.847</td>
<td>14</td>
</tr>
<tr>
<td>Immobile time (%)</td>
<td>-0.113</td>
<td>0.574</td>
<td>27</td>
<td>-0.058</td>
<td>0.843</td>
<td>14</td>
</tr>
<tr>
<td>Moving mins</td>
<td>0.086</td>
<td>0.668</td>
<td>27</td>
<td>0.132</td>
<td>0.653</td>
<td>14</td>
</tr>
<tr>
<td>Moving time (%)</td>
<td>0.113</td>
<td>0.574</td>
<td>27</td>
<td>0.058</td>
<td>0.843</td>
<td>14</td>
</tr>
<tr>
<td>No. Immobile phases</td>
<td>0.048</td>
<td>0.811</td>
<td>27</td>
<td>-0.138</td>
<td>0.637</td>
<td>14</td>
</tr>
<tr>
<td>Mean length immobility</td>
<td>-0.115</td>
<td>0.568</td>
<td>27</td>
<td>0.036</td>
<td>0.902</td>
<td>14</td>
</tr>
<tr>
<td>One minute immobility</td>
<td>0.086</td>
<td>0.671</td>
<td>27</td>
<td>0.075</td>
<td>0.798</td>
<td>14</td>
</tr>
<tr>
<td>One minute immobility (%)</td>
<td>0.093</td>
<td>0.644</td>
<td>27</td>
<td>0.440</td>
<td>0.115</td>
<td>14</td>
</tr>
<tr>
<td>Total activity score</td>
<td>-0.235</td>
<td>0.239</td>
<td>27</td>
<td>0.130</td>
<td>0.658</td>
<td>14</td>
</tr>
<tr>
<td>Mean activity score</td>
<td>-0.221</td>
<td>0.268</td>
<td>27</td>
<td>0.105</td>
<td>0.721</td>
<td>14</td>
</tr>
<tr>
<td>Mean score in active periods</td>
<td>-0.359</td>
<td>0.066</td>
<td>27</td>
<td>0.086</td>
<td>0.769</td>
<td>14</td>
</tr>
<tr>
<td>Fragmentation Index</td>
<td>0.107</td>
<td>0.596</td>
<td>27</td>
<td>0.253</td>
<td>0.383</td>
<td>14</td>
</tr>
<tr>
<td>Average wake movement</td>
<td>0.001</td>
<td>0.996</td>
<td>27</td>
<td>-0.031</td>
<td>0.915</td>
<td>14</td>
</tr>
</tbody>
</table>

±log transformed; ≠n=41 (not n=42) due to n=1 Asian participant not having a vitamin D measurement
5.3.7 Association between vitamin D status and subjective sleep parameters

Partial correlations were run between vitamin D status and the self-reported time ‘try to sleep’, sleep duration and wake time (n=42) (Table 5.12). There were no significant associations between time ‘try to sleep’, subjectively reported sleep duration or wake time with vitamin D status in either ethnic group (P>0.05). When adjusting for bone pain (n=23 Caucasian, n=11 Asian) or muscle pain (n=21 Caucasian, n=8 Asian) the results were still not statistically significant in either ethnic group. In addition, controlling for outdoor light exposure (time spent over 1000 lux) did not change the results (P>0.05). Spearman’s Rho correlations were run between vitamin D status and global scores for the PSQI (n=43) (Table 5.12). In Caucasians only, increased 25(OH)D concentration was associated with increased global PSQI score (r=0.385, P=0.047, n=27), as well as increased score for the sleep latency scale (LATEN) (r=0.439, P=0.022, n=27). This suggests poorer overall self-reported sleep quality and longer self-reported sleep latency with better vitamin D status in Caucasians. In Asians, there were no significant associations between vitamin D status and any of the PSQI subscales, or the global score (Table 5.12).

<table>
<thead>
<tr>
<th>Subjective sleep parameters</th>
<th>Caucasians</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Time ‘try to sleep’ (Dec.h)≠</td>
<td>0.151</td>
<td>0.452</td>
</tr>
<tr>
<td>Subjectively reported sleep duration (Dec.h)≠</td>
<td>-0.035</td>
<td>0.863</td>
</tr>
<tr>
<td>Self-reported wake time (Dec.h)≠</td>
<td>0.143</td>
<td>0.477</td>
</tr>
<tr>
<td>Global PSQI score±</td>
<td>0.385</td>
<td>0.047</td>
</tr>
<tr>
<td>DURAT±</td>
<td>0.284</td>
<td>0.151</td>
</tr>
<tr>
<td>DISTB±</td>
<td>0.27</td>
<td>0.173</td>
</tr>
<tr>
<td>LATEN±</td>
<td>0.439</td>
<td>0.022</td>
</tr>
<tr>
<td>DAYDYS±</td>
<td>0.264</td>
<td>0.183</td>
</tr>
<tr>
<td>HSE±</td>
<td>0.212</td>
<td>0.289</td>
</tr>
<tr>
<td>SLPQUAL±</td>
<td>0.276</td>
<td>0.163</td>
</tr>
<tr>
<td>MEDS±</td>
<td>¥</td>
<td>¥</td>
</tr>
</tbody>
</table>

n.b. ≠n=41 (not n=42) due to n=1 Asian participant not having a vitamin D measurement; ±n=42 instead of n=43 as 1 Asian subject had no vitamin D measurement; PSQI scores= Spearman’s Rho; other parameters= Pearson’s ; ¥ no data as score for MEDS=0 for all Caucasians on this scale; DAYDYS Daytime dysfunction, DISTB Sleep disturbances, DURAT Sleep duration, HSE Habitual Sleep Efficiency, LATEN Sleep latency, MEDS Sleep medication, SLPQUAL Sleep quality

Fishers exact 2x2 tests showed no significant association between bone pain and scores on the LATEN scale in Caucasians (P>0.999). However, there was a trend for a positive association between bone pain and LATEN in Asians (P=0.105) (Table 5.13). For muscle pain, there was no

---

For this analysis the LATEN scale was split into two categories to allow contingency table analysis
significant association between muscle pain and LATEN in Caucasians (P>0.999) or Asians (P>0.999) (Table 5.14).

Table 5.13: Association between bone pain score (0 or 1) and LATEN scale

<table>
<thead>
<tr>
<th>Bone pain</th>
<th>Caucasians n=23</th>
<th>Asians n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bone pain ‘Yes’</td>
<td>Bone pain ‘No’</td>
</tr>
<tr>
<td>LATEN 0-1</td>
<td>10 (43%)</td>
<td>11 (48%)</td>
</tr>
<tr>
<td>LATEN 2-3</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Fishers exact 2x2 test</td>
<td>P&gt;0.999</td>
<td>P=0.105</td>
</tr>
</tbody>
</table>

Table 5.14: Association between muscle pain score (0 or 1) and LATEN scale

<table>
<thead>
<tr>
<th>Bone pain</th>
<th>Caucasians n=21</th>
<th>Asians n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle pain</td>
<td>Muscle pain</td>
</tr>
<tr>
<td>LATEN 0-1</td>
<td>9 (43%)</td>
<td>11 (52%)</td>
</tr>
<tr>
<td>LATEN 2-3</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Fishers exact 2x2 test</td>
<td>P&gt;0.999</td>
<td>P&gt;0.999</td>
</tr>
</tbody>
</table>

5.3.8 Association between vitamin D status and PSQI scores- whole cohort

Spearman’s Rho correlations were run between vitamin D status and PSQI scores for the whole cohort of participants (n=108). Of the n=108 who had PSQI scores, n=99 also had vitamin D measurements. There were no significant associations between global PSQI scores, or any of the sub-scales with vitamin D status (P>0.05) in either ethnic group (n=99; Table 5.15).

5.3.9 Association between vitamin D status and light exposure

Pearson’s correlations were run between vitamin D status and time spent over the different lux thresholds (Table 5.16). There were no significant associations between vitamin D status and time spent (minutes) over any of the lux thresholds (P>0.05) in either ethnic group. Hence, there was no association between vitamin D status and light exposure within ethnic groups.
Table 5.15: Summary of Spearman’s Rho correlation between PSQI scores and vitamin D status - all participants in whole cohort (n=99) *

<table>
<thead>
<tr>
<th>Subjective sleep parameters</th>
<th>Caucasians</th>
<th>Asians</th>
<th>Caucasians</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>r</td>
<td>p</td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>Global PSQI score</td>
<td>0.093</td>
<td>0.432</td>
<td>73</td>
<td>-0.147</td>
</tr>
<tr>
<td>DURAT</td>
<td>0.035</td>
<td>0.768</td>
<td>73</td>
<td>-0.019</td>
</tr>
<tr>
<td>DISTB</td>
<td>0.040</td>
<td>0.734</td>
<td>73</td>
<td>0.014</td>
</tr>
<tr>
<td>LATEN</td>
<td>0.052</td>
<td>0.665</td>
<td>73</td>
<td>-0.189</td>
</tr>
<tr>
<td>DAYDYS</td>
<td>0.092</td>
<td>0.439</td>
<td>73</td>
<td>0.090</td>
</tr>
<tr>
<td>HSE</td>
<td>0.140</td>
<td>0.236</td>
<td>73</td>
<td>-0.052</td>
</tr>
<tr>
<td>SLPQUAL</td>
<td>0.027</td>
<td>0.820</td>
<td>73</td>
<td>-0.214</td>
</tr>
<tr>
<td>MEDS</td>
<td>-0.021</td>
<td>0.857</td>
<td>73</td>
<td>0.281</td>
</tr>
</tbody>
</table>

* n=99 (rather than n=108) as n=6 Caucasian and n=3 Asian women had no vitamin D status measurement. DAYDYS Daytime dysfunction, DISTB Sleep disturbances, DURAT Sleep duration, HSE Habitual Sleep Efficiency, LATEN Sleep latency, MEDS Sleep medication, SLPQUAL Sleep quality

Table 5.16: Summary of Pearson’s correlations between light exposure and vitamin D status - all participants (n=28≠)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasians</th>
<th>Asians</th>
<th>Caucasians</th>
<th>Asians</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mins&gt;100lux</td>
<td>0.165</td>
<td>0.488</td>
<td>20</td>
<td>0.255</td>
</tr>
<tr>
<td>Mins&gt;200lux</td>
<td>0.177</td>
<td>0.455</td>
<td>20</td>
<td>0.089</td>
</tr>
<tr>
<td>Mins&gt;500lux</td>
<td>0.184</td>
<td>0.437</td>
<td>20</td>
<td>0.077</td>
</tr>
<tr>
<td>Mins&gt;1000lux</td>
<td>0.048</td>
<td>0.841</td>
<td>20</td>
<td>-0.008</td>
</tr>
<tr>
<td>Mins&gt;2000lux</td>
<td>-0.025</td>
<td>0.918</td>
<td>20</td>
<td>0.074</td>
</tr>
</tbody>
</table>

≠n=28 (rather than n=29) as one participant did not have a vitamin D measurement

Partial correlations showed that when bone pain (n=19 Caucasian and n=7 Asian), or muscle pain (n=17 Caucasian and n=6 Asian) were controlled for in the analysis there were still no significant associations between vitamin D status and any of the lux thresholds in either ethnic group (P>0.05).
5.4 Results II - Matched pairs

5.4.1 Actigraphic sleep parameters

For the analysis of matched pairs, each Asian participant was matched to a Caucasian participant by age and menopausal status. There were n=15 Asians in total so 15 pairs were created. Table 5.17 illustrates details of the matched pairs. Participant characteristics for the n=15 matched pairs can be seen in Tables 5.18 to 5.20. Independent samples t-tests showed a significant difference between the Asians and Caucasians in the matched pairs for BMI, with the Asians having a higher BMI by 6.5 kg/m² (P<0.001) than Caucasians.

Table 5.17: Characteristics of matched participants (n=15 pairs)

<table>
<thead>
<tr>
<th>Pair</th>
<th>Asian n=15</th>
<th>Caucasian n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Menopausal Status*</td>
<td>Age</td>
</tr>
<tr>
<td>1</td>
<td>post</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>post</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>post</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>post</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>post</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>menopausal</td>
<td>53</td>
</tr>
<tr>
<td>7</td>
<td>post</td>
<td>64</td>
</tr>
<tr>
<td>8</td>
<td>pre</td>
<td>48</td>
</tr>
<tr>
<td>9</td>
<td>post</td>
<td>64</td>
</tr>
<tr>
<td>10</td>
<td>post</td>
<td>61</td>
</tr>
<tr>
<td>11</td>
<td>pre</td>
<td>42</td>
</tr>
<tr>
<td>12</td>
<td>post</td>
<td>64</td>
</tr>
<tr>
<td>13</td>
<td>post</td>
<td>65</td>
</tr>
<tr>
<td>14</td>
<td>menopausal</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>menopausal</td>
<td>51</td>
</tr>
</tbody>
</table>

*pre=prenopausal; post=postmenopausal ±number of days actiwatch data available

The Caucasians had significantly higher caffeine intake from coffee (P=0.051) than did the Asians, but this difference was very small (<1/2 a cup of coffee per day). There was no significant ethnic difference in age, IMD, caffeine from tea, mid-sleep time or social jetlag (Table 5.18). Fisher exact tests showed no association between ethnicity and sleep medication use, or smoking status (Table 5.19). However, there was a significant association between ethnic group and alcohol intake, with the Caucasians having a higher alcohol intake (P<0.001; Table 5.20).
Table 5.18: Participant characteristics- subjects in matched pairs analysis (n=15 pairs)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasians</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>15</td>
<td>59.7</td>
<td>10.2</td>
<td>15</td>
<td>58.3</td>
<td>7.1</td>
<td>0.666</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMD</td>
<td>14</td>
<td>11.5</td>
<td>7.1</td>
<td>15</td>
<td>12.3</td>
<td>10.4</td>
<td>0.812</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15</td>
<td>24.4</td>
<td>3.4</td>
<td>15</td>
<td>30.8</td>
<td>5.1</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine- cups of tea/d</td>
<td>15</td>
<td>2.5</td>
<td>1.9</td>
<td>15</td>
<td>2.9</td>
<td>1.1</td>
<td>0.567</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine- cups of coffee/d</td>
<td>15</td>
<td>0.7</td>
<td>0.9</td>
<td>14</td>
<td>0.1</td>
<td>0.4</td>
<td>0.051</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-sleep (MSFsc)</td>
<td>14</td>
<td>3.6</td>
<td>0.6</td>
<td>10</td>
<td>3.7</td>
<td>1.2</td>
<td>0.771</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social Jetlag (SJL)</td>
<td>14</td>
<td>0.4</td>
<td>0.5</td>
<td>10</td>
<td>0.4</td>
<td>0.7</td>
<td>0.983</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*±independent samples t-test

Table 5.19: Participant characteristics- use of sleep medications and smoking status for subjects in matched pairs analysis (n=15 pairs)

<table>
<thead>
<tr>
<th></th>
<th>Caucasians</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% Yes</td>
<td>% No</td>
<td>n</td>
<td>% Yes</td>
<td>% No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep medication user</td>
<td>15</td>
<td>0</td>
<td>100</td>
<td>12</td>
<td>17</td>
<td>83</td>
<td>0.188</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>15</td>
<td>7</td>
<td>93</td>
<td>15</td>
<td>0</td>
<td>100</td>
<td>0.309</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fishers exact 2x2 test (p value)

Table 5.20: Participant characteristics- alcohol intake for subjects in matched pairs analysis (n=15 pairs)

<table>
<thead>
<tr>
<th>Alcohol units per week</th>
<th>Caucasian n=15</th>
<th>Asian n=14≠</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 units</td>
<td>27%</td>
<td>93%</td>
</tr>
<tr>
<td>1-4 units</td>
<td>27%</td>
<td>7%</td>
</tr>
<tr>
<td>5-14 units</td>
<td>40%</td>
<td>0%</td>
</tr>
<tr>
<td>15+ units</td>
<td>6%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Fisher Exact 4x2

P_A<0.001 P_B<0.001

≠ n=1 Asian did not answer question

Indeed, 100% of Asians drank ≤4 units of alcohol per week, compared with only 54% of Caucasians.

Table 5.21 shows the actigraphic sleep parameters for the n=15 matched pairs. The results for sleep latency, sleep fragmentation, sleep efficiency and sleep duration are presented in more detail in sections 5.4.1.1 – 5.4.1.4.
For the PSQI paired data, there were only 11 pairs (n=22) as four pairs had to be excluded due to one of each pair being one of the n=4 subjects with incomplete PSQI questionnaires. Participant characteristics for the n=11 pairs can be seen in Table 5.22-5.24. Independent samples t-tests showed a significant difference between the Asians and Caucasians in the matched pairs for BMI, with the Asians having a higher BMI by 7.5 kg/m$^2$ (P=0.001) than Caucasians. There was no significant ethnic difference in age, IMD, caffeine from tea or coffee, mid-sleep time or social jetlag (Table 5.22). Fisher exact tests showed no statistically significant association between ethnicity and sleep medication use, or smoking status (Table 5.23). There was a statistically significant association between ethnic group and alcohol intake, with the Caucasians having a higher alcohol intake (P=0.05)(Table 5.24). Indeed, 100% of Asians drank 4 or less units if alcohol per week, compared with only 54% of Caucasians.
Table 5.22: Participant characteristics subjects in PSQI matched pairs (n=11 pairs)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasians</th>
<th>Asians</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age</td>
<td>11</td>
<td>64.4</td>
<td>4.4</td>
</tr>
<tr>
<td>IMD</td>
<td>10</td>
<td>11.2</td>
<td>7.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>11</td>
<td>24.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Caffeine- cups of tea/d</td>
<td>11</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Caffeine- cups of coffee/d</td>
<td>11</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Mid-sleep (MSFsc)</td>
<td>10</td>
<td>3.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Social Jetlag (SJL)</td>
<td>10</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 5.23: Participant characteristics subjects in PSQI matched pairs (n=11 pairs)

<table>
<thead>
<tr>
<th></th>
<th>Caucasians</th>
<th>Asians</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% Yes</td>
<td>% No</td>
</tr>
<tr>
<td>Sleep medication user</td>
<td>11</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Smoker</td>
<td>11</td>
<td>9</td>
<td>91</td>
</tr>
</tbody>
</table>

*Fisher’s exact 2x2 test (p value)

Table 5.24: Participant Characteristics subjects in PSQI matched pairs (n=11 pairs)

<table>
<thead>
<tr>
<th>Alcohol units per week</th>
<th>Caucasian n=11</th>
<th>Asian n=10≠</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 units</td>
<td>36%</td>
<td>90%</td>
</tr>
<tr>
<td>1-4 units</td>
<td>18%</td>
<td>10%</td>
</tr>
<tr>
<td>5-14 units</td>
<td>36%</td>
<td>0%</td>
</tr>
<tr>
<td>15+ units</td>
<td>10%</td>
<td>0%</td>
</tr>
<tr>
<td>Fisher Exact 4x2</td>
<td>P&lt;0.046</td>
<td>P&lt;0.034</td>
</tr>
</tbody>
</table>

≠ n=1 Asian did not answer question

5.4.1.1 Sleep latency

Mean (±SD) for sleep latency was similar between Caucasians (0.2 ± 0.2 Dec.h.) and Asians (0.4 ± 0.3 Dec.h.) (Figure 5.14). A paired t-test showed no significant difference between Asians and Caucasians for sleep latency (t=0.663, P=0.518).

![Figure 5.14: Sleep latency in each matched pair](image.png)
5.4.1.2 Sleep fragmentation

A paired t-test showed a significant difference between Asian and Caucasians for fragmentation index ($t=2.732, P=0.016$), with Asians having a mean (±SD) fragmentation index of $35.4 \pm 8.9$ units, compared with $26.5\pm7.6$ units (Figure 5.15). This equates to Asians having 33% higher fragmentation index than Caucasians.

Figure 5.15: Sleep fragmentation in each matched pair
These differences in sleep fragmentation were observable from inspection of the Actograms of the matched pairs (see Figures 5.16-5.17 for two example postmenopausal pairs, and Appendix AG for the Actograms of the $n=12$ postmenopausal pairs).

5.4.1.3 Sleep efficiency

A paired t-test showed a significant difference between Asian and Caucasians for sleep efficiency ($t=3.746, P=0.002$; Figure 5.18), with the Caucasians having a mean (±SD) sleep efficiency of $86.1 \pm 4.9\%$, compared with $79.1 \pm 6.6\%$ in Asians. This equates to Asians having 7% lower sleep efficiency than Caucasians, on average. 100% of both Asians and Caucasians had sleep efficiency less than 95%, and 100% of Asians and 73% of Caucasians had sleep efficiency less than 90%.

Figure 5.18: Sleep efficiency in each matched pair
5.4.1.4 Sleep duration

Mean (±SD) for actual sleep time was similar for Caucasians (6.9 ± 0.5) and Asians (6.4 ± 1.1), with a paired t-test showing no significant difference for actual sleep time (P=0.098; Figure 5.19).

Figure 5.19: Actual sleep time in each matched pair
Figure 5.16: An example of one postmenopausal matched pair (Pair 7; Asian on left, Caucasian on right), whereby the Asian participant has increased sleep disturbance throughout the night.
Figure 5.17: An example of one postmenopausal matched pair (Pair 9; Asian on left, Caucasian on right) whereby the Asian participant does not have increased sleep disturbance throughout the night. (Note the prayer period around 5am for the Asian Actogram).
5.4.2 Subjective sleep parameters

Details of the subjective sleep parameters (from the sleep diaries) in the matched pairs (n=15 pairs), by ethnicity are shown in Table 5.25

Table 5.25: Summary of subjective sleep parameters- participants in matched pairs (n=15 pairs)

<table>
<thead>
<tr>
<th>Subjective sleep parameters</th>
<th>Caucasians</th>
<th>Asians</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Time 'try to sleep' (Dec.h)</td>
<td>15</td>
<td>23.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Subjectively reported sleep duration</td>
<td>15</td>
<td>8.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Self-reported wake time (Dec.h)</td>
<td>15</td>
<td>7.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Global PSQI score ≠</td>
<td>15</td>
<td>4.1</td>
<td>3.4</td>
</tr>
<tr>
<td>DURAT</td>
<td>15</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>DISTB</td>
<td>15</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>LATEN</td>
<td>15</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>DAYDYS</td>
<td>15</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>HSE</td>
<td>15</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>SLPQUAL</td>
<td>15</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>MEDS</td>
<td>15</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

≠n=11 for PSQI scores as n=4 Asians had missing PSQI or incomplete data on PSQI a=independent t-test; b=Mann Whitney U test. DAYDYS Daytime dysfunction, DISTB Sleep disturbances, DURAT Sleep duration, HSE Habitual Sleep Efficiency, LATEN Sleep latency, MEDS Sleep medication, SLPQUAL Sleep quality

Independent t-tests showed no significant differences in time ‘try to sleep’, subjectively reported sleep duration or wake time by ethnic group. Mann Whitney U tests showed a significantly higher global PSQI score in Asians (P=0.013; mean (±SD): 8.4 ± 5.4 for Asians vs. 4.1 ± 3.4 for Caucasians). A significantly higher score for the sleep duration (DURAT) subscale was also seen in Asians (P=0.002; mean (±SD): 1.7 ± 1.2 for Asians vs. 0.3 ± 0.6 for Caucasians). There were no significant ethnic differences in the other subscales. In Table 5.26, are the equivalent data for just the n=11 pairs in the PSQI analysis. The results for this analysis (Table 5.26) were very similar to that of the n=15 matched pairs (Table 5.25), so will not be discussed in detail again here.
Table 5.26: Summary of subjective sleep parameters- participants in PSQI matched pairs (n=11 pairs)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasians</th>
<th>Asians</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time ‘try to sleep’ (Dec.h)</td>
<td>23.5 ± 0.5</td>
<td>23.3 ± 0.8</td>
<td>0.609a</td>
</tr>
<tr>
<td>Subjectively reported sleep duration (Dec.h.)</td>
<td>8.0 ± 0.5</td>
<td>8.1 ± 1.4</td>
<td>0.827</td>
</tr>
<tr>
<td>Self-reported wake time (Dec.h)</td>
<td>7.5 ± 0.7</td>
<td>7.4 ± 1.0</td>
<td>0.884a</td>
</tr>
<tr>
<td>Global PSQI score</td>
<td>3.7 ± 2.7</td>
<td>8.4 ± 5.4</td>
<td>0.013b</td>
</tr>
<tr>
<td>DURAT</td>
<td>0.2 ± 0.4</td>
<td>1.7 ± 1.2</td>
<td>0.002b</td>
</tr>
<tr>
<td>DISTB</td>
<td>1.1 ± 0.5</td>
<td>1.6 ± 0.7</td>
<td>0.193ab</td>
</tr>
<tr>
<td>LATEN</td>
<td>0.8 ± 1.0</td>
<td>1.2 ± 1.1</td>
<td>0.438b</td>
</tr>
<tr>
<td>DAYDYS</td>
<td>0.6 ± 0.7</td>
<td>0.9 ± 1.1</td>
<td>0.606b</td>
</tr>
<tr>
<td>HSE</td>
<td>0.6 ± 0.8</td>
<td>0.8 ± 1.2</td>
<td>0.699b</td>
</tr>
<tr>
<td>SLPQUAL</td>
<td>0.6 ± 0.5</td>
<td>1.3 ± 1.0</td>
<td>0.101b</td>
</tr>
<tr>
<td>MEDS</td>
<td>0.0 ± 0.0</td>
<td>0.9 ± 1.3</td>
<td>0.151b</td>
</tr>
</tbody>
</table>

#n=22 for PSQI scores as n=4 Asians had missing PSQI or incomplete data on PSQI, so n=4 Caucasian partners also excluded; a=independent t-test; b=Mann Whitney U test. DAYDYS Daytime dysfunction, DISTB Sleep disturbances, DURAT Sleep duration, HSE Habitual Sleep Efficiency, LATEN Sleep latency, MEDS Sleep medication, SLPQUAL Sleep quality.

5.4.2.1 Self-reported sleep duration, time ‘try to sleep’ and wake time

Paired t-tests showed that there was no significant difference by ethnicity in subjective sleep duration (P=0.784), time ‘try to sleep’ (P=0.462), or wake time (P=0.732) (Figure 5.20) in the n=15 matched pairs.

5.4.2.2 PSQI global scores

On the PSQI, 46% more Caucasians than Asians scored as no sleep disorder (score ≤5)(Table 5.27) Mean (±SD) for global PSQI score were significantly lower in Caucasians (4.5 ± 3.7) than Asians (8.2 ± 5.2). Wilcoxon matched-pairs signed rank test showed a significant difference between Asian and Caucasians for global PSQI score (P=0.051; Figure 5.21).
Figure 5.20: Subjective sleep duration (A); time ‘try to sleep (B) and wake time (C) in the n=15 matched pairs

Table 5.27: n and % scoring as no sleep disorder (PSQI≤5) (n=11 pairs)

<table>
<thead>
<tr>
<th>Group</th>
<th>n (%) scoring ≤5 on PSQI (no sleep disorder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>9/11 (82%)</td>
</tr>
<tr>
<td>Asians</td>
<td>4/11 (36%)</td>
</tr>
</tbody>
</table>

Figure 5.21: Global PSQI score in each matched pair (n=11 pairs)
5.5 Discussion

5.5.1 Ethnic differences in light exposure

This chapter provides novel data on light exposure and rest-activity cycles in South Asian women. It has been shown here that South Asian women spend less time in light intensities greater than 100 lux, 500 lux and 1000 lux per day than Caucasian women. The 500 lux threshold corresponds to a well-lit interior environment (e.g. office), whereas 100 lux corresponds to a more dimly lit environment and 1000 lux to an outdoor environment. The fact that the Caucasians showed a significantly more time being spent in light levels over 1000 lux suggests they had an increased outdoor light exposure relative to Asians. There was also a trend for the Asian women, to spend less time per day over the other light thresholds calculated (>200lux, >2000lux) than the Caucasian women, however these results were not statistically significant. Within ethnic groups there was a trend for premenopausal women to have lower light exposure than postmenopausal women. Thus, the difference was likely due to a combination of factors relating to both ethnicity and menopausal status. These could include biological factors (e.g. health, genetics, ageing) as well as social and cultural factors (e.g. housing, work and family responsibilities, sleep routines in the home). Moreover, all the statistically significant group differences were between the postmenopausal Caucasian and the premenopausal Asian groups.

For the daily time (hourly lux) profiles, there was a non-statistical trend for a higher light exposure in the two Caucasian groups than the two Asian groups throughout the day. Most of the light exposure in all the four groups came during the period when vitamin D can be produced (12:00 h to 16:00 h). For all four groups there was less light exposure in the morning (07:00 h to 12:00 h), although there was a trend for the two Caucasian groups to receive higher light exposure than Asians during this period. This finding suggests that Asian women may be outdoors less in the morning than Caucasian women. Time profiles were broadly similar for the rest of the day, but with a trend for a lower intensity for the Asian women throughout the day. It can be speculated from these trends that Asian women may be at risk of not gaining sufficient morning light to get the required circadian phase advance to enable entrainment to the 24 hour day. However, further research is required to investigate this further as the results were only trends but did not reach statistical significance.

It can be speculated that the above trends may be explained by the Asian women being less inclined to spend time outdoors. This might be due to cultural practices that encourage indoor activity, as well as sun avoidance (von Hurst et al. 2009). There is no previous literature to compare these results with, as no other studies to date have examined light exposure in South Asian groups. Few studies have assessed ethnic differences in light exposure. The study by Kripke et al. (2004) found
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lower light exposure in Black and Hispanic women compared with European Caucasian women (Kripke et al. 2004). The Kripke et al study (2004) found covariate adjusted mesor log10 lux was 1.14 (1.11-1.17) for European Americans, vs. 0.98 (0.92-1.05) for Hispanic Americans and 0.94 (0.86-1.03) in African Americans (Kripke et al. 2004). These ethnic differences were speculated to be due to ethnic differences in sleep disordered breathing and other non-light related factors (e.g. social factors) (Kripke et al. 2004). Similarly, the study by Jean-Louis et al. (2000) showed European Americans had a mean (±SD) for mesor lux of 559 ± 847 lux vs. 536 ± 542 lux in ethnic minority groups (Hispanics, Blacks) (Jean-Louis et al. 2000). Jean Louis et al. (2000) suggested that the minority groups showed increased circadian disregulation, which may explain their poorer sleep quality. They also tentatively suggested that social factors (such as stress) may play a role (Jean-Louis et al. 2000). It is difficult to compare these data with the results of the current study, due to lux readings being analysed by cosinor regression, which was not undertaken in this study, due to not having repeated measures data. However, the results of this chapter do agree with the results of these two previous studies in that an ethnic minority group had lower lux exposure than western dwelling white Caucasians.

5.5.2 Ethnic differences in actigraphic and subjective sleep measures

In terms of actigraphic sleep parameters, there was no difference by ethnicity or menopausal status in sleep latency. However, there was a significant ethnic (but not menopausal status) difference in sleep duration, sleep efficiency and sleep fragmentation. The NPCRA results suggested no difference in the timing of the peak and nadir by ethnic menopausal group (as shown by the similar L5 and M10 onset, but differing L5 and M10 values). There were also differences in amplitude (and relative amplitude), indicating differing magnitude of peak to nadir change in activity levels by ethnic menopausal status group. Premenopausal Caucasians had the largest amplitude, and the lowest relative amplitude was in postmenopausal Asians. The increased activity (M10) and increased amplitude of the circadian rhythm in the Caucasians could be due to the increased light exposure received by this ethnic group.

There are no previous data examining South Asian people’s sleep using actigraphic procedures or NPCRA to compare these results with. However, these results agree with that of Jean-Louis et al. (2000) who found higher sleep efficiency in non-Hispanic white participants than in Hispanic and Black participants (Jean-Louis et al. 2000). However, Jean-Louis et al. (2000) also found a shorter total sleep time and longer sleep latency in Black and Hispanic participants. In the current study, a shorter sleep time, but not longer sleep latency was found in the results for South Asian women. The slight discrepancy between our results and that of Jean-Louis et al (2000) could be due to the fact that our study used a different ethnic group (i.e. South Asian) and that our study used only
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women. Both studies used actigraphic devices, albeit from different manufacturers. A shorter sleep duration in female African Americans and Hispanic Americans than European Americans had previously been described in the literature (Kripke et al. 2004). Some ethnic differences in tau (i.e. length of endogenous circadian rhythm) have been found, with Black populations having a shorter tau than White populations (Eastman et al. 2012). This would suggest differences in sleep timing and circadian phase by ethnicity. However, these differences were not seen for the South Asian and Caucasian women in the current study.

In terms of subjective reporting of sleep quality, there was no ethnic or menopausal status difference in sleep duration or wake time. However, the premenopausal Asians had a significantly earlier time ‘try to sleep’ than the other three groups by 50-67mins (depending on comparison group). It is unclear why this would be the case. With a small sample size it is possible that the result from the premenopausal Asian group is misleading due to a small number of unusual individuals. Further research with a larger sample is required to investigate this further. For the PSQI global scores of the whole cohort, there were no significant ethnic or menopausal status difference in scores. However, when only the women who participated in the light and sleep sub-study were included in the analysis, there was a significant group difference in global PSQI scores. In this sub-sample, only 50% of postmenopausal Asians and 73% of postmenopausal Caucasians met the PSQI criteria for no sleep disorder. For premenopausal women, 100% of Caucasians, but only 25% of Asians met the criteria for being no sleep disorder. Thus, the global PSQI scores suggest that there were many disordered sleepers in the sample, and proportionately more Asians were classified as sleep disordered. There are no previous studies assessing self-reported sleep problems in South Asian groups. Although, in other ethnic groups, one study found increased self-reported sleep problems in European Americans, compared with Black Americans (Jean-Louis et al. 2001). Furthermore, in the study by Baron et al. (2010), increased self-reported excessive daytime sleepiness was seen in African Americans, as compared with European Americans (Baron et al. 2010). It is difficult to compare these studies with the data presented in the current study as they used different gender and ethnic groups. However, it does suggest self-reported sleep problems vary by ethnicity. It is not clear to what degree such self-reports reflect cultural norms for sleep quality, and how much is due to actual physiological differences in sleep. However, the results were in agreement in the current study between the self-reported sleep quality (e.g. PSQI) and the objectively measured (actigraphic) sleep parameters (e.g. sleep fragmentation) in the South Asian women.

5.5.3 Matched pairs analysis

The matched-pairs analysis supported the above findings, with an ethnic difference seen in actigraphic sleep efficiency and sleep fragmentation. There was no ethnic difference in actigraphic
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sleep latency or sleep duration, or in subjectively reported time try to sleep, sleep duration or wake
time. This matched analysis controlled for age and menopausal status of subjects, thus lending
stronger support to the other findings that Asians have poorer sleep efficiency and increased sleep
fragmentation compared to their Caucasians counterparts.

Self-reported sleep quality via the PSQI global scores was significantly different between the ethnic
groups, when only the matched-pairs were considered. This is a clinically meaningful difference, as
<5 is the commonly used cut off for no sleep disorder (Buysse 1989). These results indicate that
when both age and menopausal status are controlled for, the findings of increased sleep
fragmentation, reduced sleep efficiency and increased PSQI score in the Asians remain. As discussed
above (Section 5.5.2), the poorer self-reported sleep in the Asians is supported by actigraphic
evidence of poorer sleep efficiency and increased sleep fragmentation.

5.5.4 Regression analysis

From the regression analyses, 32% of the variance in sleep fragmentation index and 44% of the
variance in sleep efficiency were explained by ethnicity, BMI and age. This represents a moderately
large predictive ability of these variables in explaining sleep fragmentation and sleep efficiency. Of
note, however, is that ethnicity is clearly the larger predictor, explaining the majority of the variance
in both sleep fragmentation and sleep efficiency. Age is known to have a strong influence on sleep
architecture (reviewed by Espiritu 2008). Therefore, it is surprising that age did not have a stronger
influence on sleep in this analysis. A likely explanation for this discrepancy is that the age range of
the participants in the study was small (around 40 years to 70 years), thus there may not have been a
large enough range of age to show its true influence on sleep (i.e. both younger and frail elderly
adults were not included in this age range). It was also surprising that an association between BMI
and sleep was not seen here, considering the known sleep problems in persons with a higher BMI,
including sleep apnoea (Wall et al. 2010).

Previous studies in sleep research have not usually included ethnicity as a variable, so its effects
have not been previously measured. This study suggests that ethnicity is potentially a powerful
predictor of sleep efficiency and sleep fragmentation. It is not possible to say from this analysis
which aspects of ethnicity (e.g. lifestyle, genetics, physiology) are responsible, nor is it clear whether
this applies just to South Asian women and whether ethnicity is a weaker predictor in other ethnic
groups, or in men. Ethnicity and age were not predictive for self-reported data (time ‘try to sleep’,
wake time and subjectively reported sleep duration) or for actigraphic sleep duration and sleep
latency.
Light exposure, sleep and vitamin D status
The regression analyses presented in this chapter to some extent both agree with and contrast to the results of Jean Louis et al. (2000) and Kripke et al. (2004) who found that ethnicity was a significant predictor of total sleep time and sleep efficiency, as well as actigraphic sleep duration respectively. In the study by Kripke et al. (2004), actigraphic sleep duration was predicted by ethnic group (Kripke et al. 2004). As discussed above, the discrepancy between these current results and that of Jean-Louis et al. (2000) and Kripke et al. (2004) is likely due to the different gender and ethnic sub-groups used in the analyses.

5.5.5 Explanations for ethnic differences in sleep
It can be speculated that the lower sleep efficiency (and concurrent increased sleep fragmentation) seen here could be due to lifestyle and genetic differences between the two ethnic groups. For example, ethnic minority groups are over represented in urban areas (Garner and Bhattacharyya 2011). These areas may be are likely to have increased night-time light from street lighting and increased ambient noise levels, which had been found to be associated with increased risk of sleep disturbance (Jakovljevic et al. 2006). In the current study, both ethnic groups came from housing areas of similar deprivation index, making this explanation seem less plausible. However, there may be other social factors about South Asian compared with Caucasian households that are not measured by deprivation index (e.g. number of persons living in the household; adult children or elderly relatives living in home) which could lead to sleep disruption. One alternative explanation is that some of the premenopausal Asians were older than their premenopausal Caucasian counterparts and so may be closer to the menopausal transition and could be experiencing early symptoms. This could clearly disrupt sleep as the menopause is known to be associated with a variety of sleep problems (reviewed by Eichling and Sahni 2005). However, it would not explain the ethnic differences also seen in the postmenopausal women where the ethnic groups were closer in age. The postmenopausal Asian women in the current study had a higher BMI than their postmenopausal Caucasians. Obesity is known to affect sleep (reviewed by Hargens et al. 2013) and this may partly explain the ethnic differences in sleep seen here in the older women.

It must also be borne in mind that there are cultural differences in the reporting of health issues, including sleep problems. It is difficult to elucidate whether increased self-reported sleep problems in one ethnic group are due to differences in actual sleep quality, or in differing cultural perceptions of what constitutes ‘normal’ sleep. The PSQI asks for counts of events, so avoiding the more general descriptions of sleep that may be more prone to cultural bias. In addition, in this study, the Asian women also demonstrated increased sleep fragmentation, as measured objectively by actigraphy. Consequently, it is likely that their PSQI scores are reflecting this, rather than simply a cultural bias to report more symptoms.
Last, there may be differences in circadian and sleep genes between the two ethnic groups which may affect sleep. Previous studies have shown global differences in PER 3 (Barbosa et al. 2010; Ciarleglio et al. 2008) and CLOCK (Ciarleglio et al. 2008) genes, which may translate into differences in circadian timing and thus sleep processing. Overall, the most likely explanation for the ethnic differences in sleep seen in this research is a combination of social, physiological and genetic factors.

5.5.6 Vitamin D status, light and sleep quality

For actigraphic parameters, in Asians only, a significant negative association between 25(OH)D concentration and sleep latency was found. Thus higher vitamin D status in Asians was associated with shorter sleep latency, but this association disappeared when controlling for bone or muscle pain. This suggests there could be a mediating effect of bone and muscle pain in the relationship between vitamin D status and sleep latency. Previous studies support the link between sleep problems and musculoskeletal pain (Chen et al. 2011; Valrie et al. 2013). In Caucasians, adjusting for bone pain led to a significant positive association between vitamin D status and sleep latency, which had not been there previously. These results are conflicting and warrant further research. Theoretically, it would be plausible for vitamin D deficiency to prolong sleep latency. This is due to the fact that musculoskeletal pain caused by vitamin D deficiency might prevent people from falling asleep easily (and staying asleep). The conflicting associations seen here could be due to the fact that sleep latency is not reliably measured by actigraphy. No other actigraphic parameters were associated with vitamin D status in either ethnic group, suggesting that vitamin D status is not associated with sleep, at least when measured by actigraphic methods.

For subjective measures, in Caucasians only, increased 25(OH)D concentration was associated with increased PSQI score, including an increased score for sleep latency. This suggests that in Caucasians, poorer overall self-reported sleep quality and longer self-reported sleep latency were associated with better vitamin D status. This is the opposite to that found for actigraphic measures in the Asian group, where for unadjusted data, sleep latency was shorter with increased vitamin D concentration. It is also the opposite to the hypothesis that vitamin D deficiency would extend sleep latency time. It is difficult to find an explanation for these discrepant findings. Unfortunately, it was not possible to control for muscle and bone pain for the PSQI and 25(OH)D analysis as the only available test was the Spearmans Rho (due to the non-parametric nature of the PSQI data). However, correlations between 25(OH)D and bone and muscle pain were examined. In Asians only, the PSQI sleep latency scale was positively associated with bone pain scores.
Light exposure, sleep and vitamin D status

Some of the conflicting results above could be explained by the self-reported nature of the PSQI leading to a different direction of result to that found for actigraphic measures of sleep, as well as the different ethnic groups being studied. It is difficult to interpret these contradictory relationships, but they suggest that the interaction between ethnicity, method of sleep latency assessment, degree of musculoskeletal pain, vitamin D concentration and actual sleep latency may be complex. Conversely, the significant association between vitamin D status and PSQI results in the light and sleep sub-study (for Caucasians) could be a spurious one, produced by the small sample size. When PSQI scores from the whole cohort were analysed, giving increased statistical power, there was no association between vitamin D status and PSQI scores.

In terms of previous literature, there has been little on the subject of vitamin D status and sleep quality. One conference abstract reported a shorter sleep duration with poorer vitamin D status (Pande et al. 2009). By contrast, in this thesis, no relationship between vitamin D status and sleep duration was found, contradicting the above findings. Pande et al (2009) used survey data, and they did not control for musculoskeletal pain or light exposure in their analysis. It is unclear as to whether this could be the reason why the Pande et al (2009) study found an association between sleep duration and vitamin D status, whereas the current study did not. A few other studies have also assessed the relationship between sleep quality and vitamin D status. McCarty et al. (2012) found that in vitamin D sufficient patients (i.e. 25(OH)D more than 50nmol/L), Epworth Sleepiness Scores were higher in those with lower 25(OH)D in all ethnic groups (McCarty et al. 2012). However, for vitamin D deficient participants of Black ethnicity, sleepiness scores were positively correlated with 25(OH)D, which was the opposite finding to that found for other ethnic groups, and for Blacks with sufficient vitamin D status (McCarty et al. 2012). This variability in the relationship between vitamin D status and daytime sleepiness depending on participant characteristics echoes the findings of this thesis, which varied depending on the ethnic group being studied, and the method used to assess sleep latency (i.e. actigraphic, PSQI). More recently, in a study of chronic pain patients, it was found that vitamin D deficiency is more common in chronic pain patients being investigated for sleep disorders than in the general population (McCarty et al. 2013), tentatively suggesting a link between vitamin D status and sleep problems. The findings of this chapter contradict this in that no clear evidence of a relationship between vitamin D status and sleep quality was found.

Finally, there were no significant associations between vitamin D status and light exposure in either ethnic group. This was surprising considering the predicted positive relationship between light exposure and vitamin D status. A possible explanation for this would be that the women were not going outside at the correct times of day for vitamin D production. However, the results from the daily light profiles suggest the opposite, namely that most outdoor light exposure was received
Light exposure, sleep and vitamin D status during the time period when vitamin D production is enabled. In all groups, most light exposure was obtained between 12:00 h and 16:00 h, when the skin can produce vitamin D. The lack of a relationship between light exposure and vitamin D status is difficult to explain here. Also, most of the women’s time was spent indoors. From the time profiles and lux threshold data, the average light levels throughout the day was consistently under 1000 lux, and the women only spent on average 0.5 h to 1.5 h per day over 1000 lux, and 0.25 h to 1 h per day over 2000 lux.

Finally, another explanation is that the light and sleep sub-study was undertaken in autumn (September to October), not summer. More vitamin D can be produced in the summer time, due to the increased intensity of radiation, as well as the zenith angle of the sun enabling longer periods for vitamin D production. Much of the circulating vitamin D measured in the autumn may be drawn from body stores which had been produced by summer sunlight exposure. It may be that the individuals who do not go out in the sunlight in summer, may do so in autumn, when the sun is less intense, or vice versa. Importantly, the time delay between measuring the vitamin D (summer) and lux measurements (autumn) was not ideal and may explain why no relationship between vitamin D status and light exposure was found in this study. This time delay must be borne in mind when interpreting this data. Further research assessing the relationship between UVB exposure, visible light exposure and vitamin D status in different seasons is required to further investigate all the above issues.

5.5.7 Limitations

The limitations of this study include the use of actigraphy to measure ‘sleep’ parameters. At best actigraphy is an estimate of rest-activity cycles, so is not a measure of sleep per se, and has limitations due to the reliance of measurements of arm movement as a proxy for wakefulness. Particularly, actigraphy does not give accurate estimates of sleep latency, which may explain the conflicting findings in this chapter regarding sleep latency and vitamin D status in different ethnic groups when actigraphy vs. PSQI are used. Similarly, there are some practical limitations in the ability of the light sensor on the activwatch to measure light exposure. Some of these problems were overcome by using a neck cord mounted Actiwatch, rather than wrist worn, for the light readings. Neck worn monitors are less likely to be obscured by clothing, and are closer to the eye, so give a more valid measurement of light exposure to the retina than do wrist worn Actiwatches.

There are also known limitations of the use of subjective reports of sleep quality. The PSQI is a validated self-report method (Buysse et al. 1989). Nonetheless, all self-reports potentially suffer from the problems of participant memory or bias issues, participant motivation to be accurate, as well as differential understanding of the questions. In addition, the analysis used a heterogeneous
Light exposure, sleep and vitamin D status population, of varying ages (older and younger women) and a culturally varied sample of South Asian women, whose IMD scores showed they were of reasonably high socio-economic status as compared with the general South Asian population in the UK. There are no previous data to the author’s knowledge to compare the South Asian women’s results with to check concordance. However, the data reported here for the Caucasian women are in agreement with previous actiwatch data collected in community dwelling Caucasians by researchers at the University of Surrey. For example, the mean (±SEM; n) actigraphic sleep efficiency (%) reported here for all Caucasian women was 87.0% ± 0.8 (n=27) compared with 82.2% ± 2.0 (n=18) (PhD Thesis of Dr. Katarina Lederle 2010). Last, only one time period in the year was measured (i.e. September - October). The sample size for many of the parameters was low, due to the small number of South Asian women in the study. The sample size was further restricted by the requirement for good compliance with wearing the Actiwatches (i.e. 7 or more days of valid data). These limitations are discussed further in the Thesis discussion (Chapter 7).

5.6 Conclusion

The study showed that Asians spent significantly less time per day in light intensities over 100, 500, 1000 lux compared with Caucasians, with a trend for less time over 200 and 2000 lux. This difference mainly stemmed from the difference between postmenopausal Caucasian and premenopausal Asian groups, suggesting a role of both ethnicity and age. Most of the light exposure in all the four groups was received during the period when vitamin D would be produced (12:00h to 16:00h) but when circadian advance does not occur. For all four groups there was less light exposure in the morning (07:00 h to 12:00 h), although Caucasians had a trend for a higher exposure during this period than Asians. There was a non-statistically significant trend for higher light exposure in the Caucasians compared to the Asians throughout the daytime light exposure period.

In terms of actigraphic sleep measures, the Asians had a reduced sleep efficiency, shorter sleep duration and increased sleep fragmentation index compared with Caucasians. However, there was no significant ethnic difference in sleep latency, time ‘try to sleep’ or wake time. Matched pairs analysis whereby participants were matched by age and menopausal status, confirmed the reduced sleep efficiency and increased sleep fragmentation in the Asian women compared with Caucasian women.

Multiple regression models showed that ethnicity, BMI and age explained 31%-44% of variance in sleep fragmentation and sleep efficiency, respectively. However, ethnicity, BMI and age had no predictive ability in explaining sleep duration (actigraphic or self-reported), time ‘try to sleep’, wake time or sleep latency. The NPCRA results indicated differences in the magnitude of peak to nadir
Light exposure, sleep and vitamin D status change in activity levels (amplitude, relative amplitude) by ethnic menopausal status group, but no difference in the timing of the peak and nadir. Last, sleep quality, as assessed by the PSQI, was worse in Asians than Caucasians.

In terms of vitamin D, this research gives no clear evidence that vitamin D is associated with sleep quality. Although there is some suggestion that sleep latency might be associated with vitamin D status, results may vary by ethnicity, whether bone pain was a factor in this relationship and how sleep latency was measured. Surprisingly, there were no associations between vitamin D status and daily light exposure. Overall, the ethnic differences in actigraphic sleep measures seen in this analysis are likely due to a combination of social, genetic, behavioural and physiological variables, which affect sleep quality. Further research is required with a larger sample to re-confirm these results.
CHAPTER 6 Social influences on sun exposure and vitamin D production
6.1 Introduction

Sun exposure is known to be the main determinant of vitamin D production. Therefore, any factors that influence sun exposure are likely to influence vitamin D status. These include the social influences on sun exposure such as cultural beliefs, perceptions and attitudes, which may include perceptions of skin colour and beauty, and perceptions of health risk of sun exposure, which potentially vary between different ethnic groups.

Most research on sun exposure to date has been conducted in western dwelling, White Caucasian populations, with a lack of research assessing sun exposure in Muslim and Hindu populations. The few studies that have been undertaken have suggested that the latter populations show some degree of sun avoidance (von Hurst et al. 2009; Thomas et al. 2010). The little research that has been conducted has been carried out in a variety of different countries, religious and ethnic groups, and age and gender sub-populations, making generalisations difficult. Also, most research to date has used a quantitative approach via rating scales in questionnaires rather than qualitative research. Qualitative research is important to elucidate the perceptions, beliefs and attitudes, as well as the cultural norms surrounding sun exposure in South Asian women. A brief discussion will now be presented on the likely social factors influencing sun exposure in South Asian women.

A potential important factor influencing sun exposure is the influence of perceptions of beauty and skin colour. It is important to understand these beliefs, as a desire for tanning (or not) may influence vitamin D status. It is known that in South Asian (Grewal 2009), Middle Eastern (AlGhamdi 2010) and African (Dadzie and Petit 2009) populations, a pale skin tone is seen as desirable. Conversely, in White Caucasian populations, a tanned skin tone is usually seen as most attractive (Clarke and Korotchenko 2009). Despite the preference for paler skin among many ethnic minority groups, other factors may also be important in determining sun exposure behaviour. In one study, religious and cultural observations, rather than to avoid skin darkening, remained the main reasons cited for body coverage in adolescent girls with olive or medium skin tones (Fairbanks et al. 2012). Indeed, the South Asian culture contains many cultural and social practices which may reduce the available time for sun exposure, as well as the desire to do so. As members of close-knit communities, many South Asian women feel a sense of responsibility to help improve the provision of community services (‘khidmat’) (Bolognani 2013). This consists of unpaid community support, which may take up much of their free time (Hussain 2005), leaving little time for sun exposure. A need to keep to cultural traditions means older South Asian women in the UK are likely to follow to some extent a similar lifestyle to that in South Asia. In terms of sun exposure this might include avoidance of outdoor activity in the middle of the day, even though the midday sun is not as hot in the UK as in South
Social influences on sun exposure

Asia. In addition to unpaid responsibilities, half of South Asian women do paid work (Ballard 1982). This again may lead to little time being available for leisure purposes.

Some research has found that migrant South Asian populations have lower levels of physical activity than the western populations they have migrated to (Waidyatilaka et al. 2013). Also, there may be other problems in urban living environments that may influence whether South Asian women can expose skin to the sun or not. For example, an Australian study found that urban dwelling South Asian women lacked private locations to be able to sunbathe (e.g. due to being over looked in high rise flats) (Brand et al. 2008). Indeed, for South Asian women, the formal cultural and religious requirements for bodily coverage in public may mean they must have a private space to sunbathe in order to allow skin exposure.

Overall, South Asian women may be at increased risk of poor vitamin D status and low sunlight exposure due to social and cultural factors related to their ethnicity. These factors are likely to include skin tone preferences, family and work life, and religious traditional behaviours. There is a clear gap in the literature regarding sun exposure research in older South Asian women in the context of vitamin D status and exposure to light, and the health ramifications of this. It is of importance for the vitamin D field to explore the attitudes, beliefs and behaviours surrounding sun exposure in South Asian women, to attempt to improve the effectiveness of public health interventions to combat the problem of vitamin D deficiency in this population group.

6.2 Methods

Interviews were conducted in an older subset (58-67 years old) of the South Asian and Caucasian women participating in the D-FINES study 2010 (see chapter 2, Section 2.2 for recruitment information). Interviews with n=27 women (12 South Asian and 15 Caucasian, final participant age range: 58-67, Table 6.1) were undertaken between February and June 2011. They were interviewed at the University of Surrey or at the Asian Network Centres (Asians only); as the individual participants desired. Ten Asian women were interviewed at their Network Centre, and 2 Asian women were interviewed at the University. All Caucasians (n=15) were interviewed at the University.

In terms of religion, 8 of the Asian women were Muslim, 2 were Hindu and 2 were Christian/Catholic. Also, of the Muslim women, 4 were veiled (all hijab only) and 8 were not veiled.
### Table 6.1 Interviewee characteristics

<table>
<thead>
<tr>
<th>Subject±</th>
<th>Age</th>
<th>Religion*</th>
<th>Country of Birth</th>
<th>Notes</th>
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<td>Veiled, Pakistani ancestry</td>
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*#Been in UK since 2 years old; ±all names are pseudonyms

In terms of country of origin, 5 women were from Pakistan, 5 from India and 2 from other countries. Therefore, the Asian women were from a wide range of diverse religious and cultural backgrounds, and this was considered when the data were interpreted. They lived in geographical areas with a reasonably low deprivation index, being classified largely from the lower band of the middle class. The limitations of this sample will be discussed further in Chapter 7 (Section 7.3).

Two of the South Asian and 3 of the Caucasian women had also previously taken part in an investigation of vitamin D production using sunbeds, run by the University of Surrey. This involved three sessions of sunbed usage and measurement of vitamin D status. Therefore, 5 of the women had more experience of sunbeds. Only 1 Caucasian and no South Asians had used a sunbed prior to the sunbed research study.
Interviews were conducted in English and lasted between 45 mins and 1 hour, depending on the respondents’ ability and willingness to continue the interview (see appendix W for interview schedule). For all Caucasian participants, English was their first language, and for all Asian participants, English was their second language. Interviews were audio tape recorded, with participants’ consent. Interviews were fully transcribed, some with the aid of Mrs Helen Vart (University of Surrey). Transcription and interviews were run concurrently. Nvivo 9 (QSR International, Australia) was used to aid the data analysis. Initial coding and analysis of the first 14 interviews yielded a comprehensive and diverse array of themes. The second set of 13 interviews was then coded, using the coding scheme that had been produced during the analysis of the initial 14 interviews. A small number of additional codes were added during the second half of the coding process, but most codes related directly to the themes already identified, and no new major themes were produced. This suggests theoretical saturation had been reached by the middle of the transcription process.

6.3 Perceptions of tanning

The first main theme arising from the qualitative analysis was that of perceptions of tanning. It is known that preferred skin tone is likely to impact on vitamin D deficiency. This is because a preference for a tanned appearance is likely to lead to increased deliberate exposure of skin to the sun, particularly without sun cream. Although burning reduces vitamin D production, it is likely that sun exposure undertaken in order to obtain a tan will result in a higher concentration of vitamin D in the body. Increased sun exposure will also increase the amount of bright light received, which may be beneficial for regulation of circadian rhythms and sleep. In contrast, a preference for pale skin, or a high degree of fear of skin cancer (or skin ageing) would be expected to encourage sun avoidance behaviour, at least in terms of keeping skin covered (clothes, sun cream etc) or seeking the shade. This would be predicted to translate into a poorer vitamin D status and lower light exposure, the latter of which could also be detrimental to circadian rhythm entrainment and to sleep quality.

Stigma has a strong effect on behaviour and may act as a catalyst for the above sun seeking or sun avoiding behaviours. Therefore, it is of interest to investigate where cultural stigma about skin colour may originate. One probable origin is that of colonial history, whereby some South Asian countries were former colonies of the British. This may influence skin colour preferences due to the former historical dominance of those with paler skin over those with darker skin in these countries. However, there may also be more modern influences at play here, such as westernised media representations. It is important to understand these influences in order to find ways to understand
Social influences on sun exposure and change sun exposure behaviour. Therefore, this section investigates the attitudes surrounding skin tone, including any social stigma, and perceived media influences on preferred skin tone among older South Asian and Caucasian women.

As expected from previous research (Grewal 2009), Asian women had a clear preference for paler skin. However, there was very little insight by most Asian participants as to why this was the case. It was seen as a cultural norm, a fact of life, and was viewed as ‘more beautiful and that’s that’. However, some South Asians also expressed the belief that it may be due to rarity of pale skin, as described by Priti below.

“I think it is just a cultural thing... maybe they have more brown people than white, so obviously whatever is in minority, they do that. (Priti)

If you go around looking for a wife for your son the first thing... Oh, she has a pale skin and she is very good, a pale skin is always very beautiful. (Amina)

The Asian women tended to use words such as ‘fair’ in conjunction with other words pertaining to attractiveness, such as ‘slim, nice and lovely’. One example was given by Gita when she was prompted on why she had expressed that fair skin was ‘more beautiful’:

Int: So, why do you think that fair skin is seen as more beautiful?

Gita: In our culture.... I don’t know. It’s always been rare, I don’t understand.... because they look beautiful, nice you know, light colour. When they wear make-up and everything, it shines on them, it’s lovely. So that’s what people think. It’s just, you know, the way people are. I don’t understand, why is that? They look good, and you, you’re so pretty, aren’t you, as well? Lovely skin and all that. What it is I don’t know.

Int: Ok, so do you think there are any social reasons in society why that was particularly chosen? Or do you think it was (trail off allowing participant to respond)?

Gita: I think why this was chosen was (that) when you are very fair, you are ‘oh, she is lovely, she is slim’. People think ‘she is slim and beautiful and fair and nice’ and this is just one of the words used, and we are used to this all the time. I don’t know. I can’t really (know); I don’t really understand this. (Gita)

10 … refers to omitted speech
Social influences on sun exposure

Gita (as well as Amina, above) also elaborated on the link to the importance of traditions (e.g. weddings) with the idealisation and desirability of the ‘pale’ bride. There was also the perception that being white helped women stand out in the crowd, and to get admiration from others. Gita’s excerpt is particularly interesting as ‘fair and lovely’ is the name of a skin lightening brand in Asia. Indeed, a few of the Asian women mentioned the use of skin lightening products by Asian women and the advertising in Asia of skin lightener creams:

...because most of people have dark skin, they don’t want to become any darker. They are (seeing) on the television every other advertisement is how to get your skin white. It is for the girls probably they look more beautiful if they are white, you know it is just the thinking. (Meera)

The above quote from Meera illustrates that in South Asia today, advertising in the media plays a role in promoting the ideal of pale skin. One or two of the Asian women expressed strong concern over the poor regulation of the safety of these creams, which were correctly perceived as potentially dangerous by these Asian women. In a similar vein, a few other Asian women expressed slightly more insight as to why darker skin was seen as less socially desirable, suggesting this was due to westernised influences. The key factor was that Asian women felt stigmatised if they had darker skin, a belief which had implications for their sun exposure behaviour. The Asian women reported that the fear of ‘going darker’ made them restrict time in the sun, even in the UK. They were aware that in Asia, outdoor manual workers get darker. Being a manual worker was seen as something ‘unfortunate’, but unavoidable because of their circumstances. Despite their sympathy for manual workers, there was a lack of explicit recognition of the stigma regarding the darker skin of these people. This is surprising as the women themselves mentioned receiving mildly derogative or surprised comments from others when they ‘got darker’.

Conversely, as expected, among Caucasians there was a preference for medium toned, golden skin (a ‘healthy golden glow’). They perceived this as both attractive and as portraying a healthy appearance:

Well, I just think in the winter you look like death warmed up sometimes, you know people are very pale. Not everybody likes having a tan, I appreciate that... (but) I like to think you look a bit more well... I do think you look healthy. (Pippa)

Caucasian women also reported that having a tan was associated with ‘feeling good’, although the reasons why were not always clearly stated. This appeared to at least be partly due to increased perceived attractiveness to others. Unlike Caucasian women, who alluded to the effort required to get tanned, among Asian women, the opposite view was expressed. Asian women felt that deliberate
darkening of skin was seen as ‘crazy’. This was an interesting concept, and suggests that intentionally darkening your skin was seen as ‘an act of madness’, and not sane behaviour. Some of their language even suggests a feeling of horror at the thought of people ‘getting darker’ on purpose. Moreover, there was also the feeling that dark skin looks ‘strange’, although it was not explicitly expressed why this was the case.

For Caucasians, the effort involved in obtaining a tan applied to both real and ‘fake’ tans, with much discussion as to whether a tan is ‘worth the effort’ or not. There was a sense that people should get ‘as dark as possible’, which implies maximum effort should be put into obtain as deep tan as you are able to achieve. However, the desired deepness of tan had clear limits, with ‘extremely dark’ skin viewed as undesirable. At this point, their opinions started to correlate with the Asian view. The Caucasians also had the view that intentional ‘over darkening’ of skin was ‘crazy’ for appearance and health reasons. Indeed, there was a clear concept of a ‘nice’ tan, which implies that ‘not-nice’ tans exist also. Indeed, as with Asians, there came a point when Caucasians expressed a clear concept of ‘not too dark’, hinting that a moderate tan is most desirable in Caucasians.

As with the stigma associated with darker skin tone in Asians, among Caucasians, there was a social stigma to having a pale skin tone after being on holiday. The Caucasian women described the social expectation that they would come back from holiday ‘with a tan’. Indeed, some women were personally disappointed if this did not happen. As did the Asians, they reported receiving mildly derogative comments from others if their skin tone did not fit what was considered desirable. This was summarised by Maria:

*My skin doesn’t tan, I don’t know why, I don’t know why. Some people seem to go this wonderful sort of copper colour and I stay white. The first time I went abroad, I went to Portugal for a month and nobody knew I had been there because it was beautiful sunny weather and I came back the same colour I went. Very odd, very disappointing....Well they just kept saying, “you haven’t got a tan, why haven’t you got a tan” and I said “I don’t know why I haven’t got a tan, I was on the beach every day, out in the sun the whole time”... (I was) exactly the same colour when I came back. Who knows why? (Maria)*

Although there was a sense that some people are more able to tan than others (i.e. some women are ‘naturally pale’), they did not feel this was necessarily recognised by others. Some Caucasian women did not fear this reaction and expressed that they were happy to come back from holiday a light colour. This showed a sense of ignoring social reactions in that they were doing what they wanted to do, as expressed by Paula:
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I started using the fake tan... I have to admit, I couldn’t be bothered in the end. I just thought ‘what you see is what you get’... I think, you get to that age sometimes, ...you do get to an age when you think, is it really worth it all? (Paula)

Indeed, Paula’s statement also demonstrates that views about desired skin colour may change with ageing, in that the social stigma may not be as feared in older women as it is in younger women. Moreover, one idea expressed by some South Asian and Caucasian women was that people ‘want what they can’t have’ and that this applies to skin colour. This is expressed clearly by Gita and Christina:

I think mankind is never satisfied or something... but we should really be... (you) should really be thankful for whatever he (God) has given to you, colour, everything, health. That’s fine, but why this is happening? I think it’s as a human being... they like to be beautiful, this or that. You have your own idea in yourself that you want to be this or you want to be that. (You) want to be dark, like in English people, why? We find it very funny and very strange! And you find this, we want to be white, that’s strange for you as well! (Gita)

I suppose if I am honest, I do think that a skin that isn’t pallid, pallid white (looks nice), you know. You have got very fair skin. I think a bit of tan does look nice. But it is a weird world we live in that I have been conditioned to think.... But on the other hand, I do not think it is vital, so what! We have got different colour skins and that is it. (Christina)

Overall, it was clear that most Asian women had a preference for a pale skin tone, and most Caucasian women had a preference for a golden skin tone. There was also some stigma in both groups associated with being seen as the ‘wrong skin tone’. These preferences, and the stigma adding pressure to the associated behaviours, are likely to increase sun exposure in Caucasians, but reduce it in South Asians, which is likely to have consequences for reduced light exposure and lower vitamin D status in Asians.

6.4 Use of sun protection

6.4.1 General use of sun protection

The second main theme arising from the qualitative analysis was that of factors influencing use of sun protection. It is known that sun protection measures reduce the risk of skin cancer. However, when correctly used these measures also block vitamin D production. Due to sunscreen not usually
being correctly applied for full sun protection (Szepietowski et al. 2004) it is unclear as to the real
effect sunscreen has on reducing vitamin D status. However, even a partial effect of blocking UVB
by sun cream may be important. This is because, in the UK, it is mostly during the hot summer
months that most people get their vitamin D requirement for the year. Therefore, our two groups of
women were asked about sun protection use. This included asking about sun creams, but also
incorporated questions about clothing, which can also block vitamin D production.

Caucasian participants discussed changes in perceptions of the need for sun protection over recent
generation. They suggested that there was a trend towards more use of sun protection in recent times than
in the decades before. They mentioned increased availability and affordability as well as the
promotion of sun cream and ‘special’ clothes for the sun. Also, there was some consideration that
childhood history was important in whether sun creams were used or not. Indeed, one woman never
used sun cream as her family did not use it when she was a child. However, most women did not
discuss childhood experiences in depth, suggesting this may have been seen as less important
perhaps than more recent experiences.

The main reason mentioned for wearing sunscreen was to stop burning. There was some concern
about anti-ageing and skin cancer effects, but less than for burning. Sun cream was only one aspect
of sun protection used. Participants also made use of longer sleeves, hats, sunglasses and sitting in
the shade to control sun exposure. Among Caucasians, there was a common concern that those who
have ‘fair skin’ should use sun protection. Moreover, there was some belief that skins were thought
to adapt to the sun and that some skins are better at dealing with sun than others. This suggests a
sense that some people will not be able to deal with the sun as easily as others, and that some of this
is beyond their control. In Caucasians, frequency of sun cream usage was lower in the UK than
abroad, where its use was common. In addition to location, usage depended on which activity was
being undertaken:

*We go to Malta on holiday and we always have sunscreen on then. I think it is because the intensity
of the sun seems stronger.* (Paula)

*Yes, yes, I do tend not to wear suntan cream in England. I mean when I was growing up the only stuff
was Ambre Solaire oil, so I mean you were virtually going out there you know.. It was ‘come and get
me’ you know... So yes, sun cream is more or less off my list now, but I know how long I can be out
there. I think that a day’s gardening at home would be fine, because you are moving around. I think
the only time I would really wear it (in the UK) is if you are actually lying in the sun reading a book
and not moving around.* (Miranda)
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Harriet: Well, unless I think I’m going to be out a lot in it, if it’s just minimal times and I’m going to be walking around then I don’t bother, but if I’m going to be anywhere for any length of time then I usually use some... Unless you’re sitting, you’re not going to get burned. Well, saying that, you can, if it’s quite windy, you still get burned can’t you. But as I say, unless I’m going to be out a longer time, then I wouldn’t put on any sun cream.

Int: So, when you’re abroad on holiday is it different or is it the same?

Harriet: No, that’s totally different because I am not going to be short bursts in the sun. After shower, cream and everything else I just put it on everywhere, absolutely everywhere.

Int: Why do you think that is?

Harriet: Because I am spending much longer out in it. But again, I don’t do any midday stuff, don’t do any sunbathing, it’s purely walking around in it. I know if you can walk around for half an hour you can get burnt. So, I just put it everywhere when we’re on holiday, because I’m out all the time. Here (UK), it’s just short trips in and out somewhere so you’re not going to have time to get burnt so much.

Perceptions of risk are known to have an influence on behaviour. In the case of vitamin D it is likely that sun exposure would be reduced if people have strong fears surrounding the development of skin cancer, ageing skin, or fears of burning. It is unclear how people manage these perceived risks, and what the impact is, if any on behaviour. It would be predicted that those women with perceptions of high risk to health will be less likely to expose themselves to the sun, unless the perceived benefits of obtaining a tan, or other benefits (e.g. vitamin D production) are strong enough to overcome this perceived risk. A strong perception of fear of the sun, coupled with a reduced desire for tanning would be predicted to be detrimental to vitamin D status. Conversely, a low perceived risk, and high desire for a tan would be predicted to lead to better vitamin D status and more light exposure.

In Caucasian women, high sun intensity and long sun exposure duration were stimuli to wear sunscreen. There was a concept of varying awareness of sun protection need between societies- (e.g. of Australia vs UK) and also changes over time (more awareness in the UK now than before). A few Asian women mentioned historical change in South Asia with younger women being seen as more concerned about adverse health effects of the sun on the skin. This was seen as part of ‘modern things’ and linked to westernisation. Overall, however, there was still a lack of awareness of sunscreen products in South Asia. Among Caucasians there was a feeling that knowledge of the sun’s effects grows with age, with less awareness in younger people. Indeed, Caucasians mentioned that less was known about adverse health effects of the sun when they were young. They mentioned some concern when they were younger about not burning due to immediate pain. However, they had
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not been concerned then about skin cancer. The main reason now for wearing sun cream was to stop burning, but with some concern about skin cancer and skin ageing also.

In this analysis, perceptions of sun exposure risk often appeared to be shaped by personal experiences, or those of close friends or relatives. Participants who had experience of close others who have had problems in the sun (e.g. due to having pigmentation problems, other health problems, or skin cancer) made them more wary of the sun. Adverse experiences of family members, including that of painful sunburn, cancer or skin problems, were associated with increased fears. Rosemary describes how her perceptions were affected by having a daughter with a skin condition:

*I realise that has really affected how I view the sun, as I spent my life with her as a young child and a teenager ‘please don’t do this, please don’t go there’, so that I think this has clouded my view as ‘oh isn’t a tan wonderful’. I have always accepted that a tan isn’t wonderful. You know, it is crazy, our skin is not made for tanning and look at the long term health problems that it can present. Australia’s record of skin cancers, yes. (Rosemary)*

These types of perceptions are likely to increase usage of sun screens, as well as other physical barriers to sunlight (e.g. sitting in the shade, wearing more clothing). This is likely to detriment vitamin D status. However, if a persons’ skin is very sensitive to the sun, then there is only a short time period when vitamin D can be produced (as once the skin is burned then no further vitamin D can be produced until the skin recovers, or at least cools down). Therefore, it may be the case that in very sensitive individuals, the usage of sunscreen is not detrimental to vitamin D status, as they burn almost as soon as they go out into the sun. High usage of sunscreen may be of more concern in individuals with a darker skin tone who can tolerate the sun to some degree, and who may lose the benefit of the sun for vitamin D production if it is completely block by sunscreen.

There were disparate views about people’s perceptions of skin cancer in the UK population. Some women said that people are not worried; others said that people are ‘terrified’. There were surprisingly common references to Australia as a comparison society, where there is a strong concern with the need for sunscreen usage (‘slip, slap, slop’) and large skin cancer fears. Women expressed the notion of bright sunshine being more ‘normal’ in Australia, so people do not ‘go mad and strip off’ as they do in the UK when the sun comes out. This ‘novelty’ of the sun in the UK may encourage behaviour whereby the skin becomes very exposed for a short period of time, and thus little vitamin D is produced (as the skin burns) and skin damage can occur. This may be detrimental for vitamin D status as vitamin D production is probably greatest during intermittent, short periods of sun exposure, at the times of day when vitamin D production is enabled.
Asian women were not as aware of the need for sun protection, perhaps because of the lower rates of skin cancer in Asia and due to their skin being less likely to burn in the sun. The lack of awareness of sunscreen use, and the lower perceived need for sunscreen back in South Asia were highlighted when discussing why people in Pakistan do not use sunscreen:

*I think they are not aware of it. It is more like awareness and they should here, I think, people are very up (on) skin cancer. (In) Pakistan, usually people who live there, they do not sit in the sun like we do, and think we do not get enough, they try to avoid it. They only sit in the sun in winter when the sun is not that hot. In summer the sun is very hot, but in winter the sun is not that hot, so you can tolerate it.* (Meera)

Indeed, this translates into a perception by Asian women of not needing to use sunscreen in the UK.

*They feel very happy when they (go out in the) sun outside. They go out for walks and they go out to seaside and over here the majority of Pakistani people here they don’t use sunblock.* (Tahira)

There also appeared to be practical reasons for the non-use of sun creams in Asia. Asian women emphasised how sun cream is expensive and not readily available in India or Pakistan. However, there was mention of sun cream becoming more available in Asia now. Some Asian women in the UK did use sun cream to stop sunburn, as did nearly all Caucasians.

Among Asians, there was much concern over the effects of sun on the skin causing a darkened appearance and the appearance of blotches of pigment on the skin. It was not explicitly stated as to whether sunscreen was also used to prevent darkening of the skin or the development of skin blemishes. However, one woman (Priti, talking about when she was in Pakistan) described how sun cream was more likely to be used if it had been recommended by a doctor or beautician:

*I didn’t use to (use suncream), but this time I went (to Pakistan) in April and that was very hot, out there the temperature was 30 or 32 °C. Somebody told me, I went to a beautician just for my facial and she said ‘you should wear a sun block for your skin’. Then I used it, otherwise I don’t.* (Priti)

Only a few of the South Asian women mentioned fears of skin cancer as a reason for using sun cream, suggesting that the cosmetic reasons, as well as issues of affordability and availability, might explain sun cream use or non-use, in addition to stopping skin burning. Overall, the low usage of sunscreen by Asian women is likely to be beneficial for vitamin D production, and thus vitamin D status. However, sun avoidance behaviour is likely to detriment vitamin D status and light exposure. Conversely, Caucasian women who wear a lot of sun cream, and apply it correctly, are likely to have...
poorer vitamin D status. From the analysis above, this appears to be those women who have fairer skin (and so burn easily), and women who have had a friend or relative with a skin condition caused by sunlight, or sensitivity to it.

### 6.4.2 Use of clothing to block the sun

Since clothing is a strong block to vitamin D production, dress style is of particular interest when investigating ethnic differences in vitamin D status. It is known that South Asian women wear a heavier, more modest style of dress, all year around. Indeed, in Muslims, arms and legs are covered in all weathers, as well as a hijab (partly veiled; i.e. hair covered but not face). Many of the Muslim women interviewed had this style of dress, although many were also more westernised and did not wear the hijab. Dress style coverage varies between different religious groups in South Asia, however it usually means more coverage than a typical western dress style. Among Muslims there is very little skin that can be exposed to the sun, usually (at most) the face, hands and feet. This means South Asian women are likely to produce less vitamin D than western dressed Caucasian women, who wear less clothing, when exposed to the same amount of sun.

The South Asian women placed a lot of emphasis on the fabrics used in different weather conditions, with different fabrics for different seasons. This meant a varying thickness of skin cover, but not different exposure of body areas to the sun, as limbs and torso were completely covered with clothing all year around. The amount of clothing worn, when and who with had clear social influences. For example, Asians reported they may wear less clothing (e.g. short sleeves) if in the garden at home with family members. Indeed, some Asians discussed how they would occasionally expose their arms or legs to the sun (e.g. if in the garden). This suggests the importance of social space, and religious appropriateness. Indeed, it is necessary that a private space is available, which is not overlooked by non-family members, in order for many South Asian women to feel able to expose their arms or legs to the sun. Some Asian women had customs and religious beliefs that allowed more flexibility, although a modest dress style was still always required in public.

Many Caucasians reported abundant use of hats, sunglasses and long sleeves if in the sun for a long duration, or if in high intensity sun, in order to stop sun burn. Temperature also seemed to be an influence on wearing longer sleeves to protect the skin from the heat. Also, they suggested this may be due to not wanting to wear ‘skimpy’ clothes at an older age. For Caucasian women, most sun exposure was on their arms, hands and face, except when on holiday. In general, the Caucasian participants did not describe dressing differently in the UK from abroad:
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*Because that is how I feel I want to dress for the tropics or a hot day in England. I don’t wear skimpy vests, I am far too old. I don’t wear T shirts, I don’t wear strappy tops, (I) live in trousers... You know the day I put a skirt on, it is quite unusual. But it certainly wouldn’t beat the idea of sitting in the sun and getting some sunlight on me.* (Christina)

Overall, for the Caucasians, dress style was a compromise between modesty (linked with ageing), need for sun protection and desired amount of skin exposure to the sun. Conversely, for South Asian women, coverage was a formal, cultural modesty requirement, and in public only the thickness of the garments was varied by season. In private, the Asian women felt able to expose slightly more skin to the sun, but this was dependent on a suitable setting for this to be culturally acceptable.

### 6.5 Sun exposure activities and routines

#### 6.5.1 Outdoor activity

The third main theme arising from the qualitative interviews was the types of sun exposure activity undertaken and the factors that influence this. It is known that in Caucasians, most of the yearly dose of vitamin D is obtained from holiday exposure abroad and from summer weekend sun exposure in the UK (Dyffey 2002). However, this may not be the case for Asians, who are likely to avoid the sun when on holiday back in Asia. Vitamin D can be obtained from sunlight while undertaking normal daily activities, if in the right season and at the right time of day. Therefore it was of interest to compare the outdoor activities undertaken by the Asian and Caucasian women in the UK.

Asians reported undertaking some outdoor work sitting directly in the sun. As discussed by Amina, this may consist of undertaking housework (e.g. vegetable preparation) in the sun, or doing gardening:

*Because you know I don’t have time because I am working. So I don’t get time and if I want to sit in the sun I prefer to go out in summer. I prefer to go every Saturday and Sunday at 9 o’clock for a walk, then I just spend two hours in the (community) centre and after that I have to come and catch up with my housework, it is the time factor. I do want to go out, what I do in the summer if I am eating, I will just go outside and eat. I sit here and if I am the only one here I go outside. If I am cutting vegetables, I just go outside. I do that kind of thing, but often I don’t get four or five hours continually just to sit and relax.* (Amina)
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Some of the Asian women appeared to use strategies to increase sun exposure, even when time was short, by doing housework or eating outside. Indeed, some Asians mentioned that they do sit outdoors, when they have the opportunity to in summer, but not in the direct sun (i.e. they sit in the shade). Therefore, Asian women may need to be encouraged to sit in the sun, even for a short time period, at times of day suitable for vitamin D production.

Asian women also said they might sit outdoors for a short time without doing work or for prayer. Some also mentioned entertaining family members in the back garden, going for a walk or going to the seaside. Therefore, a useful strategy to improve vitamin D status and light exposure may be to encourage the combination of housework activities, or family socialising, with sunlight exposure. Because of time restraints, this may be more realistic for the Asian women than would solitary, leisure focussed sun behaviour (e.g. lying down, reading etc). Indeed, like Caucasians, many older Asian women work part-time. As seen in the quote above by Amina, this may leave less time for weekday sun exposure. Of note, the Asian women reported a lot more community activities as compared to Caucasians. This community work may restrict the time available for sun exposure in the garden.

In terms of holiday choices, the Asian women reported a trend to holiday in cooler, Northern areas of Pakistan or India to ‘escape’ the summer heat. Some of the Asians interviewed also reported going to the Gulf states for a beach holiday (e.g. Dubai). Nearly all the Asian women mentioned annual visits back to Pakistan or India to see relatives. Again, these holidays could help improve vitamin D status if the Asian women spent some of the daytime outdoors in the sun, even if for a short time period.

As discussed earlier, it is important to ensure that the sun exposure occurs at the correct time for vitamin D production and light exposure. In Asia, outdoor activity was timed to avoid peak heat from the sun (i.e. they go out in mornings and late afternoon-evenings), as described by Priti and Amina:

Yeah, you don’t go out in the sun because the sun is very strong. You see you don’t go out, you stay in. That is why they finish their offices and all that quite early as well, so people can have naps in the middle of the day to avoid the sun and most people they go out in the sun in winter because that is nicer, it is a different sun. (Priti)

What we do is we start the day very early, we start the day at 6 o clock and go for one hour walk and after that I come home and I go for shopping, that kind of thing. It is not that I am sitting outside,
but we’re sitting on verandas (in the) morning sun. I can’t say that we were sitting in the sun but during the day time like say 9 or 10 o clock when the sun is quite high then we would have the morning light, but in the afternoon it is different. But people are just going around for a walk in the afternoon, that kind of thing, but we don’t expose too much. We are wearing (clothes) and they are sleeveless, so only the face and sometimes the arms are exposed to the sun. (Amina)

This morning sun exposure could be beneficial for exposure to light, but depending on the exact time, and depending on whether in Asia or the UK, it may or may not be beneficial for vitamin D production. In the UK, summer sun exposure should be encouraged from around 10am to 12 noon, ensuring both adequate exposure to morning light (for circadian phase advance) and production of vitamin D.

Like the Asian women, Caucasians reported activities like gardening, having friends around in the garden, going for days out (e.g. to the seaside) and foreign holidays. Some Caucasian women were firmly against the idea of ‘lying’ on a beach and preferred more active holidays. Indeed, some played sports and went walking. However, unlike Asians, they had a wide variety of holidays, from hot beach holidays to Africa activity holidays and cold holidays (e.g. Scandinavia). Like Asians, they also did outdoor work in the garden, or did some entertaining in the back garden.

Therefore, in Caucasians, a large number of sunny holidays (whether active or sedentary) encourage increased exposure to sunlight and improvements in vitamin D status. Like the Asians, the undertaking of outdoor work in the garden, or socialising with friends outside, is likely to improve sunlight exposure, especially if at the times of day when vitamin D production is high.

**6.5.2 Sunbeds and ‘tanning shops’**

The interviewees were asked about perceptions and beliefs surrounding sunbed usage. This is an important area of opinion to assess, as if used correctly, sunbeds are a potential therapy for vitamin D deficiency. Indeed, a recent study showed the efficacy of a mid-winter, 8 week course of sunbed therapy for vitamin D deficiency (de Gruijl and Pavel 2012). Therefore, interviewees were asked about their sunbed usage, and their beliefs and attitudes towards them.

Previous sunbed usage was low in both ethnic groups. Only two of the South Asian women and four of the Caucasian women had ever used a sunbed, all except one Caucasian, had used a sun bed only as part of the research undertaken at the University of Surrey (see Section 6.2). The two South Asian women who had taken part in the sunbed study had found it relaxing and pleasant to use. These women reported that they were not scared as they felt their skin was adapted to cope with the UVB
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rays. For instance, Tahira described the need for promotion of sunbeds to the Asian community after taking part in the sunbed research study:

No matter how much it cost, if more (Asian) people know about the sunbed, especially in winter, if people know the benefits of sunbeds in winter, especially in winter, and the cost is cheap. (Tahira)

However, Tahira did not use a sunbed again independently after the sunbed research study had finished, and in general, sunbeds were rarely used by the Asian community. The reasons given for this were that they perceived themselves as ‘dark enough already’; and they did not know many people who use them. Most of the Caucasian women interviewed had not used them, and had few friends or relatives that had. The Caucasians also suggested that sunbeds may be used more in younger than older age groups.

Caucasian women used an interesting choice of words and concepts to describe sunbeds. These, included the concept of a ‘tanning shop’, which implicitly implies ‘buying’ a tan. They also commonly mentioned financial issues such as monetary costs of using a sunbed and discontent with the money perceived to be made by shop owners. There was a clear distinction made by most Caucasians regarding tan sprays (seen as ‘ok’) and use of sunbeds (seen as ‘not ok’). This was despite both sunbeds and tan sprays being seen as ‘artificial’.

A small proportion of the Caucasian women knew women who had used sunbeds. The reason suggested for the use of sunbeds by these women was often cited as to ‘look darker’ before they go on holiday. There was a sense of getting a ‘head-start’, promoting the tanning process. This re-emphasises the importance that Caucasian participants placed on putting effort into ‘getting a tan’, and demonstrates their ‘sun seeking’ attitude. Some Caucasians also mentioned how tanning salons are used by some people to protect their skin for when they will be out all day in sun. This illustrates again the strong association among Caucasians between tanning, health and beauty. As discussed previously, both health and beauty were seen as important factors in Caucasian women’s motivation to achieve a tan, with the balance being in favour of beauty as the more important factor. Indeed, only a few Caucasian women suggested sunbeds can be used for improving health (i.e. via relaxation), but many Caucasian women suggested vanity and other appearance-based reasons as most important for using them (e.g. to hide veins in legs).

However, despite these benefits of the sunbeds, a strong degree of fear was expressed by both Asian and Caucasian women regarding the radiation generated by the sunbed. This was particularly in terms of perceived intensity of the rays and of skin cancer risk. They were fearful of the shorter term effects of the sunbed (e.g. burning), and in Caucasians this appeared to outweigh any perceived
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benefits of the sunbed in terms of resulting in a tanned appearance. Indeed, both Caucasian and Asian women reported ‘horror’ stories that they had read in the media, including illegal use by children and young teenagers. They were concerned about the possible addictive effects of sunbeds, the lack of regulation over their use and the perceived lack of education of tanning salon staff. Perhaps surprisingly, even the appearance of the sunbed was important in producing some of these fears. Indeed, they were seen as unusual and strange, even frightening. These points were well described by Rachael:

Rachael: I would avoid them, I just don’t like the look of them with all those tubes. I really don’t, I think this is something that younger people would use rather than the older generation. Why that may be I don’t know. Maybe it is that there is limited time and things where the older generation might be a bit more cautious about that. But certainly I wouldn’t use them, I have never liked the thought of them. I have always thought you were too exposed to light which perhaps wasn’t natural. (It) will have the same effects somehow, but is more dangerous in some ways because it is more concentrated I think. So, no I wouldn’t, I don’t think sunbeds are a good idea at all.

Int: Okay, is there anything else you can tell me about sunbeds. Perhaps other people’s views, as well as your own...?

Rachael: No, only the horror stories you read in the papers about young girls being allowed to use them and not use them in the way they are meant to be used, and thus suffering from damaged skins.

Caucasian women mentioned that they, and others they knew, had been put off using sunbeds when they were younger due to bad publicity. Both Asians and Caucasian women expressed a fear of skin cancer and fear of a general exposure to something ‘bad or dangerous’. There were concerns about a potential ‘hidden’ and ‘yet to be discovered’ danger, as well as being ‘too quick to be safe’. The belief that sunbed ‘rays’ were different from actual sunlight was almost universal together with the perception that these ‘rays’ were ‘not natural’ and ‘not safe’. However, some Caucasian women suggested that occasional sunbed usage, in moderation, might be ‘ok’. Many women mentioned the need for better regulation by relevant authorities and that common sense was needed with regard to the use of sunbeds, as described by Brenda:

I think if it is what you want, go for it, but don’t do it too often, sometimes it is just a base before you go on holiday. Before you get out into the sun people like to get a little bit of colour on them I think you know, but I don’t do it myself. I just did it the once, we were going abroad then and I thought to myself, I will get a little bit of a build up before I go over, not all white. But I have got common sense.

(Brenda)
The confusion in terms of the information about sunbeds that is available, and the need for proof of safety and health benefit was expressed by many participants, and illustrated by Harriet:

* Harriet: No, (sunbeds are) not natural at all. There you go, again I don’t know. It’s just cosmetic isn’t it, because there’s controversy whether it’s good for you or bad for you. I don’t know what’s proven medically, or the actual facts. I’m not knowledgeable enough to know, whether it is detrimental or not. They say it’s not good for you, but (long pause)

* Int: Who says?

* Harriet: You know when you read the papers, one says its good and another says it’s ok. You know what I mean, you just have to make your own judgements…. It’s not something that I would personally like to do.

Among the Asian women, there were reports that some of their Caucasian friends and colleagues had stopped using sunbeds due to skin cancer fears. There was a lack of belief that on balance, sunbeds had an overall benefit for health or beauty. Also, they were not aware that sunbeds are quite cheap to use, with participants suggesting they were expensive and a ‘waste of money’. The women also expressed a preference for natural sun due to other benefits (e.g. being outside). Finally, among some Caucasians, there was a belief that someone needed to be ‘desperate’ to get a tan if they used a sunbed. Pippa took part in the sunbed study and describes her experience:

* I didn’t like it. I didn’t like the fact that you were all closed in. I didn’t like that bit of it and no, it just didn’t appeal to me. I was not that desperate for a tan that I would have to go to a salon, no I didn’t like it, I wouldn’t do it again. (Pippa)

Overall perceptions of sunbeds were negative among both ethnic groups, with concerns about burning, skin cancer and the poor regulation of sunbed safety. This suggests that a lot of change would be required in the perceptions of sunbeds, alongside improvements in sunbed regulation, before they would be a viable therapy for vitamin D deficiency. Even despite these changes, it may still not be popular for South Asian women due to the problem that sunbeds cause the skin to tan.
6.6 Perceived effects of sun on wellbeing

The final theme from the qualitative analysis is that of perceived effects of the sun on physical and mental wellbeing. Beliefs about the sun and how it affects health are likely to impact on actual sun exposure behaviour, and thus vitamin D status and light exposure. Perceptions of risk for skin cancer and other age-related skin changes were already discussed in Section 6.4, so will not be mentioned again here. This section will focus on the shorter term effects of sun exposure on physical health and psychological wellbeing.

Light exposure is well known to be associated with mood, but there is also research suggesting that vitamin D status may also be associated with increased positive affect (Lansdowne and Provost 1998). This positive mood may be influential in motivating people towards outdoor behaviour, as it makes them ‘feel good’. This would then lead to continued good vitamin D status and light exposure. Therefore, it was relevant to explore with the women how the sun made them feel both physically and psychologically.

6.6.1 Physical effects of the sun

Both Caucasian and Asian women mentioned the short term adverse effects of the sun, especially dehydration and headache. Both ethnic groups had a fear of burning in the sun, particularly fairer Asians who when visiting Asia restricted sun exposure. Adverse effects of the sun are likely to shorten sun exposure time, however it is unlikely they will negatively impact on vitamin D status. This is because these detrimental effects tend to arise when vitamin D production is already compromised (when the skin is already well exposed to the sun, so is hot or burnt), so may not have a negative impact on vitamin D status, as long as they do not discourage future sun exposure.

Both ethnic groups had a concept of ‘too much sun’, or of it being ‘too hot’ or ‘too intense’. Indeed, both groups restricted their sun exposure due to fears of ‘overexposure’. This ranged from more severe effects such as bad sunstroke, burning and skin cancer to less severe effects such as overheating, dehydration and headaches. Among Caucasians, some felt that your body could adjust to the sun, and this lengthened the amount of time a person could be outside. They believed that fairer skinned people, or those who had skin that has never been exposed to the sun, were more vulnerable to the adverse effects of the sun.
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In both ethnic groups, there was some awareness of the sun for the benefits of vitamin D production. However, both ethnic groups felt most people were not aware, and that more needed to be done to increase knowledge about the benefit of sun exposure for vitamin D status:

> They (Asians) are not getting this kind of knowledge and information on how important it is to get sun exposure and vitamin D. (Priti)

> I think of all the vitamins that is the one that sort of goes under the radar to be honest, because most people know about vitamin C and all the rest of it and B, and what we need to get it, but I think vitamin D is very important really. (Maria)

> I think the main issue is because of the language barrier... people don’t understand. Older people really need some support, what is it, how it works in your body... then they keen participating, much more I think they participate. But generally, older people from South Asian communities, like in Pakistan... They don’t know about vitamin D ... one thing is the language, the other thing is the cultural thing which they don’t bare skin, exposure to sunlight, vitamin D... they just don’t do the routine ... so maybe there is an awareness in your education, will enhance their understanding. (Nita)

6.6.2 Psychological effects of the sun

Despite fears regarding the physical health effects of the sun, Caucasian women expressed feeling enjoyment when being in the sun, saying that it makes them want to do activity and makes them ‘feel brighter’. They felt it made them ‘feel good’ emotionally and physically. With reference to ‘naturalness’, many women suggested it had to be ‘real’ sun, so sunbeds were not likely to give the same good emotional feeling. They also mentioned the sun as relaxing (as well as ‘making you more active’). Concordant with these feelings, winter weather in the UK was associated with lower mood among Caucasians.

Asian women expressed that the sun in Asia was ‘too hot’ to be enjoyable for much of the day. However, like Caucasians, Asians also saw the sun as important for wellbeing. Indeed, one Asian woman reported that her daughter felt miserable in the UK due to lack of sun. For some Asians, sun was a stimulus to be active, as long as it was not over hot. Both ethnic groups mentioned how the sun gets people outside, instead of ‘being shut up inside’. They also described how the sun could be relaxing. Tahira suggested that paler persons from Pakistan may feel better in the UK as the sun intensity is less than ‘back home’, so the sun is easier to deal with:
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Very, very, pale people, normally they don’t stay outside (in Pakistan) in the sunshine for very long, they just go for a few minutes, and then come back in shade, because of the skin redness. Here (there) is much less sunlight, less in winter, like winter in Pakistan. They feel much better over here because the sun intensity is not that high, and they feel like going out in sunshine. (Tahira)

This may be a positive factor in that summer sun exposure is more tolerable in the UK than Asia due to lower sun intensity. Therefore, South Asian women may be more likely to go out in the sun in the UK than in Asia. Overall, these positive effects of sunlight on mental wellbeing are likely to increase exposure to sunlight in both ethnic groups, and thus may have a positive impact on vitamin D status and light exposure. As discussed above, women in both ethnic groups were aware that too much sun rapidly leads to detrimental effects on mental and physical wellbeing and took measures to avoid this.

As well as the desire for a tan, the psychological benefits of the sun may explain the sun seeking behaviour reported by Caucasians. Indeed, the Caucasian women pointed out how British people ‘strip off’ in the sun (‘ unlike other cultures’). This was considered when comparing the Australian and UK cultures by Emma:

*Most people just want to sit out in the sun. We went out to Australia for about seven weeks in our winter. Most people dress a lot differently than we do, they think about covering up their arms, ... whereas probably because the sun doesn’t shine as much here, as soon as it does, everybody’s stripping off (laugh). (Emma)*

Indeed, sun seeking behaviour by Caucasian women can be explained by lack of sunshine in general, as compared with people living in countries with a lot of sun:

*People with natural brown skin, I don’t think they seek the sun as much as people with a white skin races do. I don’t really know, I suppose they come from a country with plenty of sunshine so perhaps they don’t look at it with the same eye that attracts your paler skinned northern people. (Rachael)*

This sun seeking behaviour by Caucasians may be beneficial to vitamin D production in some respects, although if they are getting sunburnt then they will lose this benefit. Also, there is the sense that it is novelty which drives sun seeking behaviour in Caucasians, thus if there was more sun in the UK, sun exposure may not increase (or may even decrease), meaning this would not necessarily be beneficial for vitamin D status or light exposure. This novelty factor is likely to partly explain the lower desire for sun exposure among Asian women, as they came from a country with abundant
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sunshine, and it is not novel for them. However, this may not be the case for women of South Asian heritage who are born in the UK.

Overall, women in both ethnic groups perceived that the sun has benefits for psychological wellbeing, although they also were aware that over exposure to the sun can be detrimental to physical and psychological health. Therefore, short periods of sun exposure will allow the health benefits of sun exposure to be attained, without the bad effects of overexposure.

6.7 Discussion

This study aimed to assess the social factors influencing sunlight exposure behaviour in older first generation migrant South Asian women living in the UK. The findings of the study support previous research which has found sun avoidance in western dwelling South Asian women (von Hurst et al. 2009) as well as Muslim women living in the Middle East (Thomas et al. 2010). Most previous research on South Asian, and other Muslim or Hindu populations, has tended to use a quantitative approach (Thomas et al. 2010; Abda et al. 2012; Mousavi et al. 2011) or has been conducted outside the UK (Brand et al. 2008). This study differs from, and adds to the literature via the use of a qualitative approach to research South Asian immigrants to the UK. This methodology has enabled a deeper understanding of the perceptions, attitudes and beliefs of older South Asian women, which is valuable for producing effective public health strategies to improve sun exposure in this population group. Indeed, it is only via understanding the experiences of this group of women that such strategies are likely to be successful. This research also builds on previous work on sun exposure in societies where people traditionally avoid the sun by assessing the implications for vitamin D status and light exposure. This research uses a holistic approach to include the health effects of sun exposure, as well as the antecedents of and factors influencing level of sun exposure itself.

This research has highlighted many factors influencing sun exposure among older South Asian and Caucasian women living in the UK. The relationships between the different factors influencing sun exposure are illustrated in Figure 6.1.

6.7.1 Cultural Beliefs and Stigma

From the interviews it was established that cultural beliefs about preferences for skin tone and the stigma being associated with being the ‘wrong’ skin tone influenced perceptions of tanning, and was
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a driver of sun exposure behaviour. South Asian women showed a clear preference for a paler skin tone, in contrast to Caucasians, who desired a golden colour of skin tone.

These skin tone preferences support previous literature on South Asian (Grewal 2009), other Muslim (AlGhamdi 2010) and Caucasian populations (Clarke and Korotchenko 2009). The stigma of being a darker skin tone in Muslim cultures was described well:

*Those individuals at the lighter end of the spectrum are considered more attractive, and are therefore privileged. This privilege is embedded in ways that are not readily apparent, and the benefits, elisions and racial myths that accompany it are re-inscribed in everyday social interactions. Dark skin is stigmatised in parallel, often imperceptible, ways.* (Grewal 2009) p330

The reasons for and the perceived permanence of these ideals varied between the two ethnic groups. Among Asian women, the preference for a pale skin tone was not seen as a fashion; it had ‘always been this way’ and was linked to cultural traditions. There was little mention of health, only beauty, and the use of skin lighteners in their society was a concern for a few of the Asian women. In contrast, among older Caucasian women, attitudes to skin colour appeared to be more associated with fashion than cultural traditions, which are more likely to be subject to future change. Indeed, this is supported by previous work showing the historical changes in fashion for skin tone preference in Caucasians (Martin et al. 2009).

There has been little previous literature assessing stigma and skin tone in White Caucasians. Like the Asian women, the Caucasian women felt that having the desirable skin tone made them admired
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by others. The Caucasians appeared to have a more explicit insight as to why golden skin was seen as good, in that a person was perceived as ‘looking healthy’ and more attractive. This was also found in the study by Clarke and Korotchenko (2009), in which older Caucasian women associated a tan with health and beauty. This was unlike the Asians who appeared to articulate a vaguer notion as to why a pale skin was preferable. Indeed, the association between skin colour and status on the South Asian sub-continent is complex, due to the fact that skin colour varies by latitude, as well as by occupation (Glenn 2008). This may explain why the Asian women did not see a direct association between skin tone and perceived levels of wealth. Regardless of the reasons for the skin tone preference, both ethnic groups reported being stigmatised for being the ‘wrong’ skin tone, which had effects on their sun exposure behaviour.

These differing perceptions of tanning are likely to lead to different sun exposure behaviour preferences, with Asian women having a tendency to avoid the sun, and Caucasian women attempting to seek the sun, to a point. This is likely to lead to reduced vitamin D and light exposure among Asians, but increased vitamin D and light exposure among Caucasian women. However, as Caucasians see tans as a ‘fashion’ it is quite possible that in the future Caucasians may also become ‘sun avoiders’ if pale skin becomes fashionable. This may affect younger women more than older women, if older women are less influenced by current fashions. Conversely, among Asians, the desire for pale skin is seen as traditional and fixed. Therefore, it would require a greater degree of social change for this cultural norm to be altered.

6.7.2 Cultural practices and perceptions of risk

The actual form that sun exposure takes (sun avoidance/seeking, usage of sun protection and dress style) is particularly shaped by ethnic and cultural factors. These include cultural practices, perceptions of tanning and perceptions of risk. In terms of usage of sun protection, Asian women were less likely to perceive themselves as being at high risk of skin cancer, and seemed more likely to be afraid of cosmetic changes (e.g. tanning, skin blotches, dark patches). Skin problems such as vitiligo (light patches appearing on darker skin) is a stigmatised problem in the South Asian sub-continent, for example in India (Pichaimuthu et al. 2011). Also, although there is little research on skin cancer perceptions among ethnic minority groups, these research findings support a recent study that found that darker skinned ethnic minority groups living in western societies do not feel at risk of skin cancer (Robinson et al. 2011). Although the prevalence of skin cancer is lower in darker skinned populations than in paler skinned populations, the mortality rate is higher (Battie et al. 2013).
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The degree to which Caucasian women are fearful of sun exposure may vary by personal experience. Those who felt most vulnerable were those who had a friend or relative with skin cancer. It is known that women with a previous history of skin cancer are likely to use more sun protection (Falk et al. 2013), with perhaps more inconsistent sun protection usage in their first degree relatives (Shuk et al. 2012). Overall, it appears that risk perception in relation to skin cancer is higher in older Caucasian than Asian women, and thus may be more likely to impact on the vitamin D status of Caucasians, rather than Asians. Any perceptions of risk among Asians may be more associated with skin darkening, or blotching of skin.

The two ethnic groups held similar ideas about sunbeds. Excluding the use of sunbeds by interviewees in previous research studies, only one Caucasian woman and no Asian women had used sunbeds. The fear of danger, due to sunbed radiation, was voiced by women from both ethnic groups. This fear may be justified, since it has been found that the majority of sunbeds may be emitting illegal levels of UVB radiation in the UK (Tierney et al. 2013). Both groups saw ‘natural’ sunlight as ‘better for you’, and safer overall than use of sunbeds. There was a lack of awareness of the potential health benefits of sunbeds (e.g. vitamin D production) in both groups. Perceived financial costs of sunbeds were also relevant, but these were not accurately perceived in either ethnic group.

There is no previous literature examining sunbed use or perceptions of sunbeds by Indian or Pakistani women. However, there has been some research in Caucasians. The results of this chapter contradict the findings of an Australian study whereby Caucasians felt that indoor tanning was safer than outdoor tanning (Gordon et al. 2012) and a Canadian study where participants did not believe sunbeds were dangerous (Rhainds et al. 1999). It also contradicts work on Caucasians who believed sunbed use to be associated with increased psychological wellbeing (Dissel et al. 2009). However, these differences may be due to the age of the participants in these studies (i.e. young men and women, rather than older women). There are a lack of studies assessing attitudes to sunbeds in older persons. In the few studies that included older as well as younger adult participants (up to age 60 years), sunbed usage was lower in older persons, especially those with a family history of skin cancer (Amir et al. 2000).

The two Asian women who had used sunbeds in previous research had, perhaps surprisingly to them, a more pleasant experience than they had expected. However, they did not continue to use them after the research study had finished. This analysis suggests that this form of sun exposure was not likely to be used due to the perceptions of risk held by both ethnic groups, and the cultural ideal of a paler skin tone held by the Asian women. Sunbeds were perceived to be expensive to use, which is not an accurate perception. It is unlikely that sunbeds will be a suitable therapy for vitamin D.
deficiency in the near future, not least until tanning salons are better regulated, and UVB doses are given in a controlled manner. Also, there is a strong barrier to their use among both ethnic groups due to perceptions of their ‘dangerousness’ that will be difficult to overcome. In addition, South Asian women may not be keen to use sunbeds due to the darkening effects on their skin.

6.7.3 Activities in the sun

For South Asian women there are formal cultural norms requiring the need for modest dress style in public. There are also behavioural codes that must be adhered to, and the requirement for spending time in activities that are seen as helpful to family, friends and community. This is likely to impact on the type of outdoor activity that South Asian women undertake.

In terms of activities undertaken in the sun, Asian women seemed to undertake more sun exposure when they were undertaking a household activity (e.g. preparing vegetables) or socialising with family, rather than for solitary leisure purposes. This suggests that there is potential for Asian women to gain vitamin D by undertaking activities outdoors, when possible. This, of course, depends crucially on having a private space, where they are able to expose their skin without being seen by the general public. In one study, not having a private garden or other space to sit in was seen as a block to achieving sun exposure in South Asian women (Brand et al. 2008). This is important as this is likely to be the case for most South Asian women who are in deprived circumstances (e.g. living in high rise apartment blocks in urban areas). Some Asian women who live in more favourable housing circumstances due to increased economic wealth and social status are more likely to have access to private spaces such as gardens. However, it is likely that most South Asian women in the UK would fall into the former category. It is however encouraging that sun exposure is possible for Asian women who do have a garden which is not overlooked by strangers. Encouraging garden based activity would be a useful way to assist Asian women to increase their vitamin D and light exposure levels.

Some Asian women reported visiting Middle Eastern destinations (such as Dubai) for beach holidays. This trend (among the richer population at least) may also improve vitamin D status by allowing a small amount of sun exposure (e.g. face, hands, feet) or a moderate sun exposure (if in western dress style) in a safe environment (e.g. female only beaches). Again, this would have beneficial effects for both vitamin D production and light exposure. Encouragement of some sunlight exposure (e.g. at cooler times of day; or in winter), rather than sitting inside, would also be beneficial for South Asian women when returning to the Asian continent for visits to see relatives. This would increase sunlight exposure and improve vitamin D status. Again, this may not be possible for many Asian women who do not have the finances to return home regularly to Asia, or to go on other
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foreign holidays, which is likely to be a reasonably large proportion of the UK South Asian population.

Only a small amount of research has examined leisure activities of South Asian women living in the UK. A review study assessed all previous research on South Asian women and physical activity and found that South Asian women reported that taking time out to participate in leisure activities, such as physical activity may be viewed by their community as being selfish, as well as a fatalistic belief in health as being fixed, and fears for personal safety (Babakus and Thompson 2012). Some aspects of the findings from this chapter agree with that of the above review, including the problem of family responsibilities leaving little time for leisure, time restrictions and the problem of certain activities not being culturally appropriate for the women (Babakus and Thompson 2012). The differences between this research and that of the above review are likely to be due to different countries and South Asian sub-groups being studied and the study methods used, as well as a focus on physical activity in the studies, rather than just outdoor activity.

In contrast to the South Asian women, older Caucasian women seem to undertake a wider range of activities in the sun (e.g. gardening, sports, sunbathing) and have regular holidays in sunny destinations which encourage vitamin D production (assuming non-complete sun protection). Encouragement of further outdoor activity, with the caveat to avoid burning, would be feasible in this group to further improve vitamin D status. In particular, the Caucasians did not mention being outdoors in the morning. Caucasian women with sleeping problems, but adequate vitamin D status may benefit from switching some of their summer outdoor time from the afternoon to the morning to enable more light exposure (to enable circadian phase advance).

6.7.4 Physical and mental wellbeing

Women in both ethnic groups reported how the sun had an effect on both mental and physical wellbeing. These well-being effects seemed to influence sun exposure in both ethnic groups, with perceived positive effects increasing sun exposure, and perceived negative effects reducing sun exposure. In both ethnic groups, having a fairer skin tone, and fears of the negative consequences of over exposure (burning, dehydration, headache) restricted sun exposure. Previous research in Caucasians has also found that paler skin tone is associated with increased use of sun protection devices and sun avoidance (Hoegh et al. 1999). In Caucasians, sun exposure was especially restricted in women who had a family member with an adverse reaction to the sun (e.g. cancer, severe sunburn, skin particularly sensitive to sunlight).
Both groups felt that sunlight produced a sense of mental wellbeing. This beneficial effect of sunlight has been previously reported in the literature (Sivamani et al. 2009). Allied to the positive effects of the sun on wellbeing are those that likely stem from good vitamin D status and light exposure. Indeed, the short term benefits (e.g. good sleep, positive mood) and longer term benefits (e.g. prevention of chronic disease) of good vitamin D status and high light exposure are other positive effects that can arise from regular bursts of sun exposure.

In both Caucasians and South Asian women, there was some awareness of the benefits of the sun for vitamin D production, but there was very little knowledge of the benefits of light for sleep. Both ethnic groups felt most people were not aware, and that more needed to be done to increase knowledge about the benefit of sun exposure for vitamin D status. Very little previous research has assessed knowledge about vitamin D status and attitudes to sun exposure in Asian women. The one exception was a study in Chinese women, which highlighted a lack of knowledge about the production of vitamin D by sunlight and its health benefits, as well as discussing the problem of widespread sun avoidance (Kung and Lee 2006). The findings in this chapter demonstrated a slightly higher level of awareness of vitamin D, most likely due to participation of the interviewees in previous vitamin D research studies, and the effect of living in a different cultural setting. However, both the women in this study, and that of Kung and Lee (2006), suggest that overall lack of awareness about the importance of sun exposure for vitamin D status was low.

6.8 Conclusion

The findings in this chapter add to previous work on sun exposure in South Asian and other Muslim and Hindu populations, providing a qualitative analysis of perceptions, beliefs and attitudes, and assessing the likely impact of this on vitamin D status and light exposure. The qualitative analysis showed that cultural beliefs and stigma influence perceptions of tanning which promote the desire for a paler complexion among Asians, compared with a desired golden skin tone in Caucasian women. These views, alongside other cultural factors and perceptions of sun exposure risk, contribute to explaining sun exposure behaviour.

For older Asian women, sun exposure tended to be coupled with household work or socialising with family or friends in the garden. Among Caucasian women, sun exposure was also associated with leisure activity, or sunbathing, as well as gardening and spending time outside with friends or relatives. Caucasian women were more concerned about skin cancer and more likely to use sun protection creams than Asian women. The analysis suggested that sun exposure behaviour in turn influences physical and mental wellbeing, with regular bursts of sun exposure being associated with
Social influences on sun exposure improved self-rated well-being. This is also likely to lead to the beneficial health effects of improved vitamin D status and light exposure.

It must be borne in mind that there are some limitations to this interview study. The main limitation is that the research is restricted to South Asian women aged 57-67 years old, who have lived in the UK for a reasonable period of time, who speak reasonably good English and who are of lower middle class social status. These women had also been regular research participants in Nutrition studies, so may differ from other women of South Asian origin. These issues will be further discussed in Chapter 7 (Section 7.3.6.1).
CHAPTER 7 Discussion
7.1 Summary of Thesis results and original contribution

Chapter 1 highlighted the problem that South Asian women are known to have lower vitamin D status as well as being at increased risk of poor musculoskeletal health. From previous literature on UVB exposure, it was suspected that South Asian women spent less time outdoors, and may also have a lower light exposure. It was unknown whether South Asian women had poorer sleep than Caucasian women, and if so whether this was related to lower light exposure. The social factors influencing sunlight exposure and the likely impact on vitamin D status and light exposure had been little elucidated in South Asians, with no previous qualitative research on sun exposure in older South Asian women living in the UK. These issues are summarised in Figure 7.1; the original schematic for investigating the problem of poor vitamin D status and its health consequences in South Asian women (previously presented in chapter 1, Section 1.6.1)

Figure 7.1: A proposed schematic for investigating the problem of poor vitamin D status and its health consequences in South Asian women

This Thesis aimed to address three main gaps in the literature. First, the relative musculoskeletal health of older South Asian women as compared with Caucasian women, as well as the role of vitamin D deficiency in musculoskeletal health. Second, the light exposure of older and younger South Asian women compared to Caucasian women, their relative sleep quality and the influence of vitamin D status on sleep. Third, the social factors influencing sun exposure in older South Asians women and Caucasian women, and the likely implications of this for vitamin D status and light exposure in these two ethnic groups.
Discussion

Chapter 3 showed that postmenopausal Asians dwelling in the South-East of England have lower, but more stable 25(OH)D concentrations than postmenopausal Caucasians throughout the year. This confirmatory finding supports previous data on premenopausal women and postmenopausal published from this cohort (Darling et al. 2013; Macdonald et al. 2011). It was also demonstrated that greater seasonal cycling of 25(OH)D is associated with increased PTH concentration and with increased bone resorption. This is a novel finding as this hypothesis has not been tested before (in any population) to the author’s knowledge. This result has clinical implications for supplementation of vitamin D status (as previously discussed in Chapter 3, Section 3.4). Chapter 3 also showed that vitamin D status was lower in Asians than Caucasians in both 2006 and 2010, although there had been an increase in 25(OH)D in Asians (independent of change in supplement usage) over the four year period. This analysis adds to the literature on longitudinal change in 25(OH)D within populations, as there have been few studies in the UK (or worldwide) that has examined changes in vitamin D levels over time. This analysis is an important contribution to understanding change in 25(OH)D status over a period of years in South Asian women, who are currently underrepresented in national UK surveys of vitamin D status (e.g. NDNS). Unfortunately regression models were unable to assess the predictors of change in 25(OH)D over the four year period, most likely because the change was very small.

Chapter 4 of this Thesis demonstrated that South Asian women have a similar, or higher vBMD than Caucasian women (depending on bone site). Overall bone size is smaller in South Asian women but with adaptations (e.g. increased cortical thickness relative to size) to attempt to improve bone strength. Despite these adaptations, bone strength is still reduced compared with Caucasian bone. This is a unique finding in that bone geometry and strength (via pQCT methodology) had not previously been measured at any bone site in older South Asian women. Also, for the tibia it had not been measured before for any age sub-group of South Asian women. The finding that vitamin D status is associated with some mass, size and strength parameters in Caucasians, but only with radial trabecular density in Asians is also novel. No previous research had correlated pQCT measures of bone geometry and strength with vitamin D status in older South Asian women. Last, in terms of muscle function, it was found that Asians had slightly poorer performance than Caucasians on some measures of muscle function (e.g. grip strength, tasks of daily living) but not in other measures (e.g. stand to walk test). Vitamin D status was only associated with stand to walk test results, and in Caucasians only. This is novel work in that previously there were little data on muscle function in South Asian women living in the UK, with no comparisons between South Asian women and Caucasian women.
Discussion

Chapter 5 showed that older and younger South Asian women spend significantly less time per day over 100, 500, 1000 lux, with a trend for less time per day over 200 and 2000 lux. Daily time profiles for light exposure are similar to their same age Caucasian counterparts, although there was a trend for less time spent outdoors at all times of day in the South Asian women. A reduced actigraphic sleep efficiency and increased sleep fragmentation was seen in South Asians as compared with same age Caucasians. These are original findings, as light exposure per se (rather than specifically UVB exposure) has not been previously investigated in any South Asian populations, anywhere in the world. Previous to this study, sleep quality had been little researched in South Asian women, with only prevalence rates of insomnia being reported (Panda et al. 2012). No previous research had assessed sleep parameters using Actigraphic (or polysomnographic) measures. Therefore, this is clearly a novel contribution to the sleep literature, and highlights a clinically important and previously neglected issue. This Thesis also tested a controversial hypothesis, that of the vitamin D-sleep connection. This was an important connection to investigate, as if vitamin D contributes to sleep difficulties, then vitamin D might be a relatively cheap, effective and safe therapy for some sleep problems. However, this Thesis found no clear association between vitamin D and sleep quality or between vitamin D status and overall light exposure.

Finally, the social factors influencing sun exposure in the two ethnic groups were elucidated in Chapter 6. This analysis suggests that cultural beliefs and stigma influence perceptions of tanning, which alongside cultural norms and perceptions of sun exposure risk, affect sun exposure behaviour. Sun exposure behaviour consists of usage of sun protection, dress style, activities in the sun, and degree of sun avoidance or ‘seeking’. This sun exposure behaviour is likely to have positive and negative effects on physical and psychological wellbeing, and also to have effects on vitamin D status and exposure to light. In turn, vitamin D status is likely to have consequences for musculoskeletal health as well as light exposure being likely to influence sleep quality. Specifically, this Thesis suggests that vitamin D status may be lowered in South Asians due to sun avoidance arising from cultural factors which encourage a preference for a paler skin tone, heavy dress style, and restrictions on sun exposure activity. This work is novel as no previous work has examined qualitatively the perceptions, beliefs and attitudes surrounding sun exposure in older South Asian women living in the UK. This analysis gives an important insight into the social mechanisms promoting sun avoidance in South Asian women. It is the first analysis (to the author’s knowledge) to investigate the potential influence of social and cultural norms on health via effects on vitamin D status and exposure to sunlight, as well as to suggest strategies to increase sun exposure in South Asian women.
7.2 Wider implications of the work

7.2.1 The need for improvements in sunlight exposure, musculoskeletal health and sleep quality

7.2.1.1 Musculoskeletal health and sleep problems in South Asian women

This Thesis has shown that South Asian women have weaker bone strength than Caucasian women. In the main, this is likely to be due to their smaller bone size, rather than lower bone mineral content, or lower bone density. This suggests the need for public health or clinical intervention in older South Asian women to improve bone strength. It is likely that little can be done in older women to increase bone size. Hence, interventions to prevent fracture in older South Asian women would need to focus on improving bone mineral density, or at least maintaining the bone mass that they have for as long as possible. Clinical and public health interventions may need to be made earlier, or in a different way in this group, as compared with other ethnic groups with a larger bone size. More research is clearly required to assess the dynamics of bone tissue ageing in South Asian women, in order to help reduce fracture risk in this group. When clinicians and policy makers are assessing fracture risk, the small bone size of the South Asian women must be considered. It must also be borne in mind that DXA scans, which ignore bone size, may not be applicable for judging fracture risk in South Asians, especially if Caucasian reference ranges are used.

In terms of muscle function, the South Asian women in our study scored slightly lower than the Caucasians on the muscle function tests. Some of the reduction in grip strength and small reduction in walking speed may be due to a smaller muscle and skeletal size. Nonetheless, as Asians fared slightly worse on self-ratings of difficulties with tasks of daily living, ensuring adequate vitamin D status, some increased physical activity, and a lighter body weight may help to improve physical function, and make these tasks of daily living easier. For South Asian women who are frail, interventions that have been used to improve muscle strength in older Caucasians may be of use here, but the interventions need to be tailored to take account of the cultural background of the South Asian women (e.g. by female health professionals, in a health care setting rather than a gymnasium etc.).

The lower light exposure and poorer sleep seen in the South Asian women are also problems which need to be tackled. There is a need for encouraging more light exposure, particularly in the morning (to advance circadian phase) and between 10am-3pm (when vitamin D production is enabled). It is likely that for both ethnic groups, regular and short bursts of light at varying times of day are
necessary to expose a person to enough light sufficient to both make vitamin D and to entrain circadian rhythms. This is in contrast to less frequent and longer bursts at one time point, which increases the risk of not getting enough morning light or vitamin D, or both.

South Asian women may also benefit from culturally appropriate information and help with encouraging good sleep practices (i.e. ensuring room is dark, correct temperature, avoiding caffeine before bed etc). This information would need to be distributed in ways that are accessible to the South Asian community (e.g. in doctor’s surgeries, Asian media, Mosques etc) and in the appropriate languages. These good sleep practices may help to improve sleep and reduce musculoskeletal fatigue to some degree in South Asian women. Still, it is likely that the key to improving musculoskeletal health and sleep quality is by tackling the central problem of low outdoor exposure, which contributes to low light exposure and low vitamin D status.

7.2.1.2 A vicious cycle?

Before discussing specific strategies for improving sunlight exposure in South Asian women, it is important that the inter-connections between different factors are examined, specifically with respect to the potential vicious cycle which may be occurring in South Asian women. The value of the multi-disciplinary approach of this Thesis is clear here, with regards to the ability to synthesise data from different subject areas, leading to a more holistic view of the problem than would be achievable if the different subject areas were studies alone. The benefits also lie in being able to suggest practical solutions based on this holistic view.

The proposed vicious cycle is illustrated in Figure 7.2. This model would predict that increases in sunlight exposure, with ensuing benefits for light exposure, sleep quality and vitamin D status, is likely to enhance wellbeing in South Asian women. The reduced fatigue and musculoskeletal problems which an improvement in vitamin D status and light exposure may bring could lead (instead) to a beneficial positive feedback loop which improves sleep, increases outdoor activity, and increases sunlight exposure in turn. Increased sunlight exposure to light may also improve mood and overall functioning. However, increasing sunlight exposure in South Asian women in the UK is likely to be a challenge, due to the social and cultural factors encouraging sun avoidance.
7.2.2 Targeting barriers to change for sunlight exposure in South Asian women

Barriers to behavioural change can be classified as those at the micro level (intrapersonal) and those at the macro level (local community and societal) (Fitzgerald and Spaccarotella 2009). First, a discussion will be presented of the intrapersonal factors influencing behavioural change (7.2.2.1), followed by a discussion of the social, cultural and community level factors (7.2.2.2).

7.2.2.1 Intra-personal factors

Intrapersonal factors include the person’s knowledge, religion, level of education, age, health status, personal preferences and beliefs (Fitzgerald and Spaccarotella 2009). Some of these factors (e.g. age) cannot be changed, and others not easily changed (e.g. health status, personal beliefs). Health education aims to target the knowledge factor. Health educational programs promoting the health benefits of sunlight exposure and vitamin D status, especially for musculoskeletal health and sleep quality, may be needed to help inform at risk groups, such as South Asian women. This idea is not new, with a recent review examining the problem of vitamin D deficiency in South Asia, with recommendations for such education about the need for adequate sun exposure (Masood and Iqbal 2008). Still, the results of this Thesis will be useful in informing the format that this education could take. For instance, this Thesis suggests that a focus on the mental wellbeing associated with
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moderate sun exposure may be beneficial, as this was found to be a strong motivator in the women interviewed.

7.2.2.2 Social, cultural and community level factors

This Thesis suggests that health education programs need to take into account the social, cultural and community level factors which affect the behaviour of South Asian women. These factors include cultural norms, availability of resources within living areas and social support. In South Asians, one of the cultural norms is that pale skin is perceived attractive. This perception appears to be so embedded in the belief system of the South Asian women that it would be difficult to change. Promotion of the health benefits of the sun could be made to South Asian women, in an accessible way (as discussed above). Still, a focus on health benefits may not be strong enough to overcome this cultural ideal of attractiveness. However, in the older women (i.e. 65 years and over), it is possible that a focus on the health benefits might be more fruitful than in the younger women (i.e. young to middle-aged), as they are more likely to already be suffering from musculoskeletal health problems. Many of the older Caucasian women in the study said that they were less motivated by attractiveness now than when they were younger (i.e. they were not motivated to get a tan for reasons of attractiveness). However, this was not so explicitly mentioned in the Asians. Perhaps an emphasis on the fact that very short periods of sun exposure may improve vitamin D status and light exposure in Asian women (e.g. exposing skin for 10-15 minutes per day, without causing a large degree of skin darkening) may be useful in reducing sun avoidance.

The key point is that these messages are communicated in an accessible and culturally acceptable way to the South Asian women. Due to the importance of social and cultural factors in targeting barriers to change, these strategies are more likely to be successful if the social support networks for older South Asian women are included. This may include providing information and guidance to the friends and relatives of these women, as well as other carers (e.g. in care-homes). Friends, relatives and the community may be able to support South Asian women’s strategies to increase sun exposure (e.g. encourage family activity outdoors rather than inside, to go with them for a walk; help out with home or community responsibilities so there is more time for sun exposure; organise trips out for the elders of the community). It is important that family, friends and the community are supportive in order for the women to start, and maintain a program of increased sunlight exposure. Local South Asian women could be trained to impart sun exposure advice to older women in the community. This approach has been successfully implemented in other public health initiatives, (e.g. the training of lay workers to educate others in nutritional guidance (Kennedy et al. 1999)).

7.2.2.3 Public health initiatives
More public health initiatives are required to support increased sun exposure in older women. The formal requirement for a modest dress style in public is not amenable to change, therefore all strategies need to comply with this regulation. The social analysis presented in this Thesis suggested that many of the Asian women were happy to expose skin to the sun in their own back gardens, and enjoyed doing so on summer days when out in the garden with family, or when doing a household task (e.g. preparing vegetables). In the interviews, the Asian women were quite receptive to the acceptability of sun exposure in a garden as long as it was private. For South Asian women who do not have a garden or other private space (e.g. due to living in high rise flats), one very useful strategy would be the provision of ‘safe’ spaces in which to expose skin to the sun. Other useful initiatives may include a policy on increasing sun exposure for both South Asian and Caucasian elders in care homes. The challenge for all initiatives is to encourage some direct sun exposure, rather than always sitting in the shade, and to encourage sunlight exposure both in the morning (for circadian phase advance) and when vitamin D is produced (10am-3pm).

An alternative to vitamin D supplementation is the use of sunbeds, which have been shown to improve vitamin D status, even when used in controlled doses (de Grujil and Pavel 2012). However, the findings of this Thesis suggest sunbeds are unlikely to be acceptable to both ethnic groups, at least until sunbeds are adequately regulated by the appropriate authorities (e.g. government bodies), their efficacy and safety are tested, and perceptions of their safety altered accordingly. This is not likely to be the case in the near future. For the Asian women, there is also the barrier of not liking the tanning effects of sunbeds.

### 7.2.3 Supplementation of vitamin D

One other possible strategy for improving vitamin D status is vitamin D supplementation. Around half of the older South Asian women in the 2010 cohort took vitamin D supplements. Most of these women had been prescribed these supplements (in conjunction with calcium) by their GP due to the vitamin D deficiency found as a result of the 2006 D-FINES study. However, these women are unlikely to be representative of all South Asian women in the UK, who if suffering from the symptoms of vitamin D deficiency may need to be encouraged to visit their GP for vitamin D testing, or to purchase vitamin D from a supermarket or chemist. Although the South Asian women in this study were consuming adequate intakes of calcium (around 750 mg/d), it must be borne in mind that many South Asian women in the UK are likely to not be consuming as much calcium, particularly those consuming vegetarian and vegan diets. Therefore, a policy of vitamin D supplementation also requires awareness of the need for calcium supplements in women with poorer intakes of calcium, either as a separate supplement or part of a combined supplement with vitamin D.
The fact that only half the South Asian women who had taken part in the 2006 study, were still not taking supplements, despite almost all of them having vitamin D deficiency suggests that there is a large barrier to taking vitamin D supplements, even in individuals who take part in vitamin D research studies. There is a need for a targeted education program, appropriate for the South Asian women to understand (e.g. in appropriate languages), and in a place which is culturally acceptable and convenient for them to attend (e.g. in a mosque, or community centre, GP surgery etc.).

The optimum amount of vitamin D supplementation for at risk groups, such as South Asians is still not clear. The current UK recommendation of 10 micrograms per day (DOH 1991) could be an initial target, which could be revised if the UK recommendations change in the future. The Scientific Advisory Committee on Nutrition (SACN) are currently undertaking a Full Risk Assessment for vitamin D status in the UK, with revised vitamin D recommendations to be published in 2015.

It has been long debated, for all ethnic groups, whether vitamin D should be supplemented all year around, or just in the months when vitamin D cannot be synthesised in the skin (i.e. October to May in the UK). The argument has been presented in this Thesis that year round supplementation of vitamin D may not be as good for bone health as just supplementing in winter when no skin synthesis of vitamin D occurs. This is due to the possible detrimental effect of a seasonal fluctuation in vitamin D levels. Therefore, in individuals who have a larger degree of sun exposure (e.g. Caucasians) it may be better to supplement only in the winter to ‘blunt’ the seasonal change in vitamin D status. On the other hand, in people who do not show seasonal variation in vitamin D status, such as South Asian women who tend to have low sun exposure, it may be effective to supplement all year round.

One problem with vitamin D supplementation is that, unlike sunlight it will not increase the amount of light that South Asian women receive. Also, supplementation does not promote physical activity, which has other health benefits. As a result, it should be seen that moderate sun exposure is likely to be more beneficial than vitamin D supplements. The use of vitamin D supplements alone may be useful for those incapable of getting outdoor light, due to very restrictive social circumstances or medical debility. Good sleep hygiene practices (as discussed above) in conjunction with vitamin D supplementation may also be effective in decreasing musculoskeletal fatigue and improving sleep quality of South Asian women.

The key point from this Thesis is that it is preferable to obtain vitamin D from outdoor sunlight rather than vitamin D supplements (or sunbeds) to improve vitamin D status. Increased outdoor
exposure is likely to have additional benefits for South Asian women’s sleep and psychological wellbeing, over that of vitamin D supplements alone.

7.3 Critical evaluation of the study and areas for future work

7.3.1 Study recruitment

As an approach to recruitment for the 2010 study, contacting the original 2006 participants was a successful one. This was achieved by undertaking presentations to the various Asian women’s groups at their own centres and to the Caucasian groups at the University. Recruitment information for 2010 was successfully combined with the presentation of 2006 study findings. This method, combined with mail-outs, led to around one third of the original cohort attending the new 2010 study. Frequent liaison with the Asian women’s centres was also successful in enabling the recruitment of Asian women who had not previously taken part in the D-FINES study.

Despite these recruitment measures being in place, for the 2010 data there were still low subject numbers in the younger Caucasian group, and both Asian groups. As emphasised throughout this Thesis, has had a large influence on the statistical power and generalisability of the datasets. There was not sufficient time and budget during the research project to recruit larger numbers of participants, particularly Asian women. In future, time and funds permitting, more investment needs to be made in recruitment measures targeted at Asian women (e.g. holding recruitment ‘fairs’ in places where Asian women frequent, using ‘Asian media’, contacting more Asian women’s centres, or GP surgeries with a high number of South Asian patients). Also, numbers of premenopausal women recruited (to the light and sleep study) were low. In future research, more premenopausal Caucasian women need to be targeted, perhaps by recruiting more of the University population, or using local women’s groups, GP surgeries or social media strategies. Particularly, GP based recruitment has the benefit of being able to contact certain sub groups to increase the numbers of women with certain characteristics (e.g. younger women, or Asian women of all ages).

It must be borne in mind that volunteers taking part in the light and sleep and the interview sub-studies may differ from those not taking part. Participation in ‘extra’ studies may be biased to include participants that differ in motivation, time availability, number of social commitments and health from those taking part in only the main study. This must be considered when interpreting the data from the sub-studies. Similarly, for the comparison of vitamin D status over the four year period (2006-2010) (Chapter 3, Section 3.8), there was the problem that 2006 volunteers returning in 2010 may differ from those who did not return. This problem was investigated by assessing the data using
all the women providing data in both years, as well as doing a paired analysis (i.e. analysing only the women who attended in both years). This successfully enabled an estimated as to the effect the differing participation in the two years was having on the data.

7.3.2 Study protocol

The study protocol was outlined in Chapter 2, (Section 2.2.2) and in Appendix D so will not be reiterated here. The protocol used in the study sessions was successful. Nonetheless, in some cases the participants had to leave the session before the questionnaires were completed. Therefore, there was not enough time to complete the questionnaires during the session. In future research, the number of questions could be reduced to allow better completion, as it was sometimes difficult to get questionnaires back from participants through the post, leading to missing data. It would have been useful to have the study questionnaires (Appendices F,H,I,J,K) printed in South Asian languages, as this would have helped those with less ability in English. Some of the Asian women volunteered to help translate material for the other Asian women in the same study session, which aided the completion of questionnaires. Perhaps, having more interpreters to cover study sessions, as well as pre-translated study materials, would be helpful in future. This would enable the recruitment of more Asian women who have less ability in spoken and written English, making future samples more representative of the UK South Asian population as a whole.

7.3.3 Musculoskeletal health and vitamin D status

The pQCT measurements were successful, giving valid data for both the tibia and radius. With the pQCT data that has already been collected further analysis can be undertaken using specialist software. For example, the polar distribution of mass in the two ethnic groups can be calculated. This gives more information about the shape of the bone, and how this likely impacts on strength. Also, the pQCT dataset from the older women in 2010 can be compared with a dataset from premenopausal women obtained at the same time and included in the Thesis of Dr Ohood Hakim. In collaboration with Dr Hakim it would be valuable to ascertain the differences in the bone geometry of premenopausal and postmenopausal South Asian women.

In terms of collecting new data, a more comprehensive examination of South Asian and Caucasian bone structure could be achieved by use of HR-pQCT technology. This higher resolution scan would enable a more detailed examination of the micro architecture of the bone tissues, and any ethnic differences in this. The increased resolution of the pQCT scans enable an examination of the plates, rods and struts of the trabeculae, as well as the size of the trabecular spaces, and features of the bone cortex. These measures would provide even more detail about the likely strength of the bone in South Asian women.
A limitation of the musculoskeletal data was the use of a partially validated questionnaire (Appendix F). Most of the items from the questionnaire came from the NIH Study of Osteoporotic Fractures (N.I.H. 2003). However, this questionnaire did not cover all the aspects needed and extra questions were added. Due to the limited time available for the study to take place there was not sufficient time to validate these new questions formally. The subjects did find the questionnaire reasonably easy to complete, although they found it difficult to distinguish between muscle and bone pain, and the site of the pain. This would be expected as it is a known fact that pain is experienced by everyone differently and is a difficult sensation to describe.

The main limitation of the analysis of seasonal ‘cycling’ of 25(OH)D on sPTH and sCTX was that the findings of this work are generalisable to Caucasian and South Asian women living in the South of the UK, but may not be generalisable to other ethnic groups. This is due to potential differences in vitamin D metabolism that may affect the metabolic response of 25(OH)D, sPTH and sCTX to seasonal change. It must be borne in mind that this was a restricted sample of South Asian women, and other South Asian women in the UK may originate from different areas of Asia, thus may differ genetically from those in this sample. More research is now required as to how the relationship between seasonal fluctuation and bone markers varies between individuals who have different genetic polymorphisms influencing either bone biology or vitamin D metabolism.

Due to cost considerations, serum CTX measurements had been performed for participants who had a full data set for 2006. This meant only women who had attended and gave a blood sample on all 4 sessions in 2006 had been analysed for sCTX. As a result, there could be some bias in that women who had not attended all sessions were not included in this analysis. Therefore, those women with problems which limited their study participation (e.g. lifestyle commitments or health problems) may not be as represented as those without. On the other hand, it did mean that it was a repeated measures analysis, thus there was less bias between seasons in the analysis, as each season included the same women.

7.3.4 Assessment of diet, physical activity, lifestyle and UVB exposure

The diet diary used in the study is shown in Appendix G. The limitations of diet diaries are well known, with inaccurate reporting potentially leading to an under or over estimation of food intake. These issues were discussed in Chapter 2 (Section 2.3.7), so will not be re-iterated here. Due to the potential effects of under and over reporting on the data, this Thesis examined both energy-adjusted and non-adjusted values for vitamin D and calcium intakes. Thus, the effects of under and over reporting were considered in the analysis. The regression analysis compared change in vitamin D
intakes over two occasions, so it was assumed that the level of over and under reporting was equal in both years within individuals and non-adjusted values were used. In future, it may be best to use energy adjusted data in the regression models, in case this assumption is not a valid one.

In terms of physical activity assessment, the participants reported that they found the physical activity questionnaire (Appendix K) difficult to fill out, with some reporting that they got confused with the meaning of the questions. Perhaps an improvement would be, time and resource permitting, if more investigators could help the participants fill out the answers for this questionnaire. Alternatively the questionnaire could be re-designed and piloted to assess the design and clarity of the questions.

For sunlight exposure measurements, the dosimeter badges were returned reasonably well, but there were some issues. As found in the 2006 study, some badges were lost, put through the washing machine or simply not returned within the time frame of the study. Perhaps an improvement to study scheduling would be to invest more investigator time in chasing up lost badges. It is difficult to assess participant noncompliance for the dosimeter badges, as it is not easy to assess how much sunlight exposure they had, and compare this with the result on the badge itself. Last, there are known problems with dosimeters in terms of variance in readings dependent on where the badges are placed on the body. In both 2006 and 2010 this was standardised, with participants being asked to place it on the lapel of their outermost garment, and try to ensure that it did not become covered. Therefore, it is assumed that this was unlikely to be a between years confounding factor in this study.

7.3.5 Light and sleep sub-study

One of the advantages for the light and sleep sub-study was the availability of a well validated scale (PSQI) for assessing self-reported sleep (Buysse et al. 1989). However, the PSQI only assesses the previous month’s sleep, so it has limited validity in assessing longer term sleep. In this study, Ramadan ended a few weeks before the sleep and light sub-study started, so it was not valid to base their questionnaire scores on the sleep of the Muslim women in the previous month. This is because participation in Ramadan is known to temporarily alter sleep-wake cycles (Roky et al. 2001). For that reason, all the South Asians (even those who were not Muslims, for standardisation purposes) were asked to assess their usual sleep, not the previous month’s sleep. Ideally, a longer gap would be given so the sub-study was not run so near to Ramadan, but it was difficult to avoid both Ramadan and the change in clock time from GMT and DST (end of October), which also causes temporary disruption to sleep-wake cycles. A compromise was made so that the study was run a few weeks after Ramadan, but ended the day before the clock change.
Actigraphy is a validated method for studying rest-wake cycles (Jean-Louis et al. 2001). Polysomnography is the ‘gold standard’ method but has limitations which meant it was not suitable for this study (see Chapter 2 Section 2.3.4). As with all Actigraphic studies, there was some non-compliance with the Actiwatches which needs to be borne in mind when assessing the results of this study. Noncompliance issues included removal of the watch at night, the retrospective filling in of sleep diaries and Actiwatch records, and some missing data from sleep diaries and Actiwatch records. As described in Chapter 2 (Sections 2.2.3.2 and 2.2.3.3) measures were taken to remove periods of time when the watch had been removed, or where there were artefacts caused by the temporary malfunction of the watches (i.e. ‘spikes’). In addition, all women with less than 7 days of actigraphic data were removed from the analysis. These measures helped reduce the impact of the noncompliance, but slightly reduced subject numbers for the analysis. The reduction in available participants with valid data for analysis was 5/42 participants for the sleep analysis and 2/47 for the light analysis. The participants were advised to try and ensure that the neck worn Actiwatch did not become covered, in particular ensuring that the light window was exposed at all times. This meant it had to be worn over the outer clothing, especially in the South Asians who tended to wear heavier clothing.

It is important to bear in mind that the PSQI and the actigraphic method were designed to assess sleep in populations who sleep in one session (i.e. go to bed and sleep through to the morning and then get up). The PSQI and Actiwatches could therefore be considered ethnocentric. This is likely to have impacted on the validity of the sleep measures for some of the Asians, who have a planned awakening in the early hours. Morning prayer time, (approximately 4-5am) means South Asian Muslims deliberately set their alarm to wake up, and then they go back to sleep. The Actiwatch program may interpret this awakening as a more disrupted sleep. Prayer time tended to be around 15 minutes at the most, so was not likely to affect the results to a large degree in itself, but there may be fragmented sleep around this prayer period (i.e. a second sleep latency in falling asleep the second time). However, in this dataset, inspection of Actigrams showed a fragmented sleep throughout the night, not just around the prayer period (see Appendix AG). Nonetheless, it may be worth considering this in the future when using Actigraphy or PSQI with Muslim groups. Perhaps modified forms of these analysis techniques need to be produced, as has been done for NPCRA analysis of patients with dementia (Van Someren et al. 1999). Alternatively, the sleep period could be assessed in both ethnic groups for just up to prayer time as well as after prayer time. This would assess whether the sleep efficiency and fragmentation index are only different after the prayer time, or whether their sleep is different throughout the night (and is not necessarily related to the prayers). Overall, such issues need to be considered when analysing data from different ethnic and religious groups.
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Subject numbers were small which made it difficult to accurately compare the sleep quality of older and younger women in this study. Some of the results for the premenopausal Asian group relied heavily on a small number of individuals. Further analysis in a larger sample of women would enable further investigation of the results of this Thesis. Also, it would be interesting to assess the light exposure and sleep quality of other sub-groups of South Asian populations, such as South Asian men and children.

7.3.6 Interview sub-study

7.3.6.1 Sampling of South Asian women

One of the main limitations of the interview analysis was the sample of women it was drawn from. First, the ethnic composition of the South Asians was very diverse. The majority were Muslims, mostly from Pakistan, but a few from East Africa. There were also some Hindus, originally from India. This heterogeneous sample makes generalisations to each particular religion difficult. Second, the women were all highly motivated women who had been followed up as a research cohort since 2006, so are unlikely to be completely representative of all South Asian and Caucasian women in the UK. Third, both ethnic groups were of moderately high socioeconomic status, and lived in area of reasonably low deprivation index. The South Asian women all spoke basic English well, which again suggests they are not completely representative of all older South Asian women in the UK. This may limit the generalisability of the results to the lower band of the middle class South Asian women who have been resident in the UK for many years. Due to the above factors the findings of this Thesis are likely to be a slightly biased (i.e. optimistic) scenario for making generalisations about the health of the South Asian group. This may be especially true with respect to the number of holiday days that were reported during the interviews. In both the South Asians and Caucasians, number of holiday days abroad per year is likely to be much higher in this sample compared with the general population. Despite the good English language ability of the Asian women on average, there were still some difficulties with the expression of complex ideas. This may impact on the quality of the qualitative data obtained, as it was difficult for many of the women to express in detail their thoughts and experiences. In future, using an interviewer who is fluent in the interview’s native language would help to increase the complexity of ideas expressed.

The South Asian sample was from limited geographical areas in the UK (i.e. South East), including specific towns where there was a high concentration of South Asian people (e.g. Woking, Kingston, Croydon). Due to chain migration the individuals in each of these areas likely originated from similar areas of the countries they came from. Consequently, each of the different town and cities offered a different religious and cultural group (e.g. Hindu, Muslim, Christian). Between towns the
Asian women were of very heterogeneous background, but within towns, the women are likely to be of very similar ancestral (i.e. genetic and cultural) descent. This reflects the complex nature of the identity and origin of voluntarily migratory populations. For this Thesis, it means that there is both a diversity (between areas) and similarity (within areas) in the characteristics and genetics of the South Asian population. There was also a large diversity in dress code (including wearing of a veil, or not and proportion of bodily coverage with clothing) within and between religious groups. Further research on a larger number of more homogeneous subjects would allow further investigation of social factors within each of these specific South Asian groups. It would also be valuable to assess younger South Asian women living in the UK and assess how they are similar or dissimilar to their Caucasian counterparts, and the older South Asian population.

7.3.6.2 Interview structure and content

The time of one hour was chosen for the interviews of this study. After conducting the interviews, it was not felt in this study that extra time would not have added extra information to the analysis. Participants were very fatigued after 1 hour, especially those with English as a second language (i.e. the South Asian women). It may have been more fruitful to run a second interview on a separate occasion, rather than extend the time of the session. Some questions were asked about vitamin D but were not used in the eventual analysis due to a lack of detailed information given by the participants. This, the interviewer felt, used up some valuable time which could have been spent further examining sun exposure, and is likely to be a problem of trying too broad a scope. In future, restricting the scope of the interview to a smaller number of topics would be recommended.

7.3.6.3 Interviewer-Interviewee interaction effects

One last problem in the interview study was that of the interviewer being from a different ethnic group from the South Asian interviewee. This may facilitate objectivity on the part of the interviewer, as they are not directly a part of the culture being examined. Conversely, it may be that they have difficulty truly understanding the interviewee’s experiences (Reis and Judd 2000). Although, conversely, on occasion this can also facilitate openness on the part of the interviewee (Rhodes 1994). The interviewer may also have stereotypes about the ethnic group, which need to be overcome to get an objective interview and analysis. The language barrier may also make interviews quite difficult to conduct, and can make it difficult to access the participants true experiences in a good detail. This problem could be overcome in future studies by using an interviewer from the same ethnic group. However, this may also cause other problems such as the interviewer being too close to the subject matter, and being unable to be objective about what is being said due to too much personal knowledge of what is being communicated leading to confusion of own experience with
that of participants (Archer 2002). Here, the interviews being part of a PhD being conducted by a single researcher, it was not possible for the interviewer to be chosen, so for the interviews with the South Asian women it was fixed as a Caucasian interviewer and South Asian participant. Also, the interviewer aimed to increase objectivity by the use of the participant’s actual words in the coding process, which helps ground the analysis in the data (Charmaz 2006).

7.3.6.4 Future social and cultural research in South Asian populations

Areas for future qualitative work could include further elucidation of the social factors that may influence musculoskeletal health and sleep (e.g. physical activity levels, the influence of chronic health problems such as obesity and diabetes). Also, other types of personal reporting could be undertaken, such as focus groups to examine the issues surrounding sun exposure in a group setting. Alternatively, a discourse analysis of media items (South Asian and mainstream media) that discuss vitamin D, skin lightening, or sun protection advice would be insightful. This type of analysis could offer further information about the social influences on South Asian and Caucasian women and would be a useful supplement to the data produced here. Further quantitative methods with a social focus could also be applied. For instance, surveys into sun exposure behaviours or awareness of vitamin D could be undertaken, as done previously in Chinese women v(Kung and Lee 2006).

7.4 Thesis conclusion

This Thesis aimed to assess the biological and social influences on vitamin D status, light exposure, sleep quality and musculoskeletal health in UK dwelling South Asian and Caucasian women. It demonstrated that South Asian women living in the UK have a lower vitamin D status, lower light exposure, poorer musculoskeletal health and poorer sleep health than same-age Caucasian women. It also suggests that vitamin D status and sunlight exposure may be lower in South Asians due to sun avoidance arising from cultural factors which encourage preference for a paler skin tone, heavy dress style, as well as discouraging sun exposure as a leisure activity.

These findings suggest the need for clinical and public health intervention in South Asian women to improve vitamin D status, light exposure, musculoskeletal health and sleep quality, as appropriate. It is likely that a vicious cycle of poor health and low sunlight exposure is in existence in the South Asian women. Low sunlight exposure and low vitamin D status, may lead to musculoskeletal fatigue and poor sleep, which in turn is likely to reduce outdoor exposure, reinforcing the cycle. Interrupting this vicious cycle by improving vitamin D status and the use of indoor exercise may help improve health by improving fitness and reducing musculoskeletal fatigue. However, this will not necessarily improve sleep quality due to the adverse effects of lack of outdoor light on circadian
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entrainment. Therefore, it is important that sunlight exposure is increased, preferably in the form of outdoor activity.

Tackling this problem will require targeting barriers to change in South Asian women, perhaps using a health education approach and encouraging support from friends, family and community. It will be of importance to make sun exposure more appropriate for South Asian women (e.g. combining with other activities, including visits to family or friends, ensuring an appropriate place for sun exposure). Importantly, although supplementation with vitamin D has obvious health benefits, this Thesis suggests that it is also important that South Asian women receive more sunlight, to improve sleep health and overall wellbeing.

The main limitations of this work are the heterogeneous nature of the South Asian group, in that they came from a wide variety of religious and cultural sub-groups. They were also from households of reasonably low deprivation index, had resided in the UK for a long period of time and had good command of the English language. As a result, they are unlikely to be typical of the whole South Asian population in the UK, and may present an optimistic picture in comparison with the South Asians who come from more disadvantaged circumstances. Also, due to small subject numbers for the South Asian women, many of the analyses throughout the Thesis were underpowered. It would be useful to investigate some of the findings further in a larger sample.

Despite these limitations, this Thesis has made a novel contribution in terms of examining the musculoskeletal health of older South Asian women in more detail than had been undertaken previously in the literature. It has also, for the first time, examined overall light exposure and sleep quality in younger and older South Asian women, as well as assessing whether there is an association between vitamin D status and sleep quality. This Thesis has added to our knowledge about the social factors influencing sun exposure in older South Asian women living in the UK, and it is unique in that it has translated this information into preliminary strategies to increase sunlight exposure in this group. If successful, these strategies could be useful in improving sunlight exposure, vitamin D status, and musculoskeletal health and sleep quality in older South Asian women dwelling in the UK.
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