Interaction between Vitamin D Supplementation and Sunlight Exposure in Women Living in Opposite Latitudes (The D-SOL Study)

Marcela Moraes Mendes
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Submitted for the degree of DOCTOR OF PHILOSOPHY

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2019
DECLARATION OF ORIGINALITY

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ABSTRACT

Vitamin D deficiency is associated with detrimental effects on bone health and is currently a major global public health issue, with increasing prevalence in both low and high latitude locations. Vitamin D can be synthesised in the skin via sunlight exposure as well as ingested through diet. This study aimed to investigate the interaction and relative contribution of vitamin D supplementation and individual sunlight exposure in raising vitamin D levels above recognized vitamin D cut-off points for deficiency/sufficiency, throughout winter, in ethnically identical adult women living in opposite latitudes. Within two parallel randomized controlled trials (RCT), 135 Brazilian women, (England, n=56, 51°N; Brazil, n=79, 16°S), were randomized to receive daily 15 μg vitamin D₃ supplements or placebo, for 12 weeks. Oral vitamin D supplementation of 15 μg daily was significantly effective compared to placebo at raising 25(OH)D concentrations over winter, regardless of latitude, and response was dependent on initial 25(OH)D concentrations. Individual UV radiation level was strongly correlated with 25(OH)D concentrations. In both latitudes, supplementation prevented the seasonal concomitant increase in plasma parathyroid hormone (PTH) levels. This research shows: 1) an optimal vitamin D status for bone health around 70-80 nmol/l; 2) the required UV radiation to achieve this status was 1.5 SED; 3) the vitamin D dietary intakes required to achieve these serum levels are 4.5 µg/d at a low and 37 µg/d at higher latitude respectively, with a lower intake of 12 µg/d sufficient to achieve 50 nmol/l in high latitudes. The strength of these results is the novel analysis that directly links human in vivo individual sunlight radiation, increased vitamin D intake and 25(OH)D concentrations, within two parallel RCTs in opposite latitudes. This study demonstrates that a daily supplement of 15 µg vitamin D₃ is an effective strategy to significantly raise vitamin D concentrations throughout the winter months in adult females, with important implications for bone health through the concomitant lowering of PTH, regardless of latitude.
ACKNOWLEDGEMENTS

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_Greatly appreciated PhD and D-SOL study supervision, guidance and support, and thesis proofreading from:_

Professor Susan Lanham-New (University of Surrey)
Dr Kathryn Hart (University of Surrey)
Dr Patrícia Borges Botelho (University of Brasília)

_Statement of contributions_

*Support with participant recruitment*: Juliana Bertazzo (Brazilian Embassy in London), Abep-UK (Association of Brazilian Postgraduate students and Researcher in the UK), Emanoelly Pires and Giovana Caixeta (University of Goiás).

*Trial visit procedures*: Dr Andrea Darling, Dr Taryn Smith, Saskia Wilson-Barnes, Dr Philippa Gibsion and Hajrah Mukthar (University of Surrey); Sáskia Vaz, Amanda Oliveira, Lara Nabuco and Marília Bohnen (University of Goiás).

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LIST OF PUBLICATIONS, CONFERENCE ABSTRACTS AND PRESENTATIONS

Peer-reviewed publications


Book Chapters


Published abstracts in conference proceedings

*Oral presentation at the National Osteoporosis Conference, Birmingham, December 2018. Awarded a Research-Based Abstract Award based on scientific merit of the abstract.*

*Poster presentation at Nutrition Society Summer Conference, University of Leeds, July 2018.*

*Oral presentation at Nutrition Society Irish Section, University of Ulster, June 2018.*

*Poster presentation at the 7th Brazilian Congress of Integrated Nutrition (CBNI), São Paulo, Brazil, June 2017.*

*Oral presentation at Nutrition Society Summer Meeting, King’s College London, July 2017.*

Poster presentation at Nutrition Society Summer Meeting, University College Dublin, Ireland, July 2016.


Abstract selected for Premium Presentation and awarded a Young Investigator Award based on scientific merit of the abstract.

Conference presentations

“Overview of the D-SOL study design: a multicentre study with two parallel-randomized control trials (RCTs) on vitamin D supplementation and sunlight exposure in adult women.”

Invited to give a presentation at the Institute for Aging Research Seminar, Harvard Medical School, Boston, USA, September 2018.


Poster presentation at the 21st Vitamin D Workshop, Barcelona, Spain, May 2018.

Mendes, M. M., Darling, A.L., Hart, K., Morse, S., Murphy R.J. and Lanham-New, S.A. Impact of urban living, including lifestyle and environmental factors, on vitamin D status for UK ethnic populations: pilot analysis of D-FINES and D-SOL databases.

Poster presentation at the 21st Vitamin D Workshop, Barcelona, Spain, May 2018.
“Factors affecting vitamin D status in Brazilian women living in England – baseline results from the D-SOL study.”

**Junior Speaker at the Mini-Symposium on The Impact of Ethnicity on Risk of Diet-Related Diseases Rank Prize Funds Meeting, Lake District, February 2018.**


*Poster presentation at International Symposium of Nutritional Aspects of Osteoporosis, Hong Kong 2017.*

“Vitamin D deficiency and influential factors in Brazilian women living in Southern England.”

*Invited to give a presentation at the Human Nutrition Research Symposium, University of Surrey, October 2017.*

Invited speaker chosen, together with Dr Louise Wilson, to represent the Nutrition Society One-day International Symposium at the Mega Nutrition Conference, São Paulo, Brazil, August 2017 including 3 talks:

1. “An overview of the latest scientific evidence for the role of vitamin D In non-skeletal health”
2. “Influential factors on vitamin D production and maintenance”
3. “Does sunlight abundance suffice to maintain adequate vitamin D status? A review of the prevalence of vitamin D deficiency and influencing factors in tropical countries, with a focus on Brazil.”


*Oral Presentation at the Doctoral College Conference, University of Surrey, July 2017.*

“International Work Opportunities in Nutrition.”

*Invited keynote Speaker at the Nutrition Student Conference at the University of Goiás, June 2017.*

*Oral Presentation at KTN Early Career Researchers Event, October 2016.*


*Oral presentation Nutrition Society Student Conference 2016, Chester, September 2016.*

Mendes, M. M., Hart, K., Tripkovic, L. Botelho, P. B. & Lanham-New, S. Vitamin D supplementation in Brazilian women living in opposite latitudes (the D-SOL study)

*Oral presentation at the Oxbridge Conference for Brazilian Studies, Health and Ecology Panel, University of Oxford, May 2016.*


*Poster presentation at Postgraduate Research Conference, University of Surrey, April 2016*

Mendes, M. M. Vitamin D supplementation in Brazilian women living in opposite latitudes (the D-SOL study).

*Poster presentation at Postgraduate Research Conference, University of Surrey, April 2016*


*Poster presentation at the 19th Vitamin D Workshop, Boston, USA, March 2016.*
LIST OF AWARDS

- Research-Based Abstract Award by the National Osteoporosis Society Conference (2018)

- Osteoporosis Conference Travel Bursary to attend NOS Conference by the National Osteoporosis Society (2018)

- University of Surrey Urban Living Themes Award (2018)

- Santander PhD Mobility Award for research placement at Harvard Medical School (2018)

- Young Investigator Award by the National Osteoporosis Society Conference (2016)

- Full 3 years PhD Scholarship Award from the Science Without Borders Program (2015-2018)

- 4th Year Extension Scholarship Award from the Science Without Borders Program (2018-2019)
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<td>1,25-dihydroxyvitamin D</td>
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<tr>
<td>AI</td>
<td>Adequate Intake</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of Covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone Mineral Content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CoFID UK</td>
<td>Composition of Foods Integrated Dataset</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CYP241</td>
<td>Cytochrome P450, family 24, subfamily A, polypeptide 1</td>
</tr>
<tr>
<td>CYP27B1</td>
<td>Cytochrome P450, family 27, subfamily B, polypeptide 1</td>
</tr>
<tr>
<td>DBP</td>
<td>Vitamin D Biding Protein</td>
</tr>
<tr>
<td>DEQAS</td>
<td>Vitamin D External Quality Assessment Scheme</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference Intake</td>
</tr>
<tr>
<td>D-SOL</td>
<td>Interaction between vitamin D supplementation and sunlight exposure in adult women living in Opposite Latitudes</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual x-ray absorptiometry</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Intake</td>
</tr>
<tr>
<td>FAO/WHO</td>
<td>Food and Agriculture Organization / World Health Organization</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IBGE</td>
<td>Brazilian Institute of Geography and Statistics</td>
</tr>
<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>ITT</td>
<td>Intention To Treat</td>
</tr>
<tr>
<td>IU</td>
<td>International Units IS</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography–mass spectrometry</td>
</tr>
<tr>
<td>MED</td>
<td>Minimal Erythemal Dose</td>
</tr>
<tr>
<td>NDNS</td>
<td>National Diet and Nutrition Survey</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>PP</td>
<td>Per Protocol</td>
</tr>
<tr>
<td>pQCT</td>
<td>peripheral Quantitative Computer Tomography</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
</tr>
<tr>
<td>RNI</td>
<td>Recommended Nutrient Intake</td>
</tr>
<tr>
<td>SACN</td>
<td>Scientific Advisory Committee on Nutrition</td>
</tr>
<tr>
<td>SBEM</td>
<td>Brazilian Society of Endocrinology and Metabolism</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SED</td>
<td>Standard Erythemal Dose</td>
</tr>
<tr>
<td>SPF</td>
<td>Sun Protection Factor</td>
</tr>
<tr>
<td>TACO</td>
<td>Brazilian Food Composition Table</td>
</tr>
<tr>
<td>UL</td>
<td>Tolerable Upper Intake Level</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet radiation</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultra Violet B radiation</td>
</tr>
<tr>
<td>vBMD</td>
<td>volumetric Bone Mineral Density</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
</tr>
<tr>
<td>VDSP</td>
<td>Vitamin D Standardization Program</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>°N</td>
<td>Latitude degrees North</td>
</tr>
<tr>
<td>°S</td>
<td>Latitude degrees South</td>
</tr>
</tbody>
</table>
Introduction

1.1 Introductory remarks

The earliest humans evolved in environments with extremely high ultraviolet (UV) radiation from the sun, in equatorial Africa, and were dark skinned. Although UV radiation has detrimental effects to our cells when in excess, it is also essential for the production of vitamin D in the skin. Living at the equator, these primitive populations therefore received high amounts of UV radiation, but the melanin in their skin – responsible for skin pigmentation – functioned as an effective natural sunscreen against the cell degradations of sunlight exposure, still allowing for vitamin D production (1–3).

As humans dispersed over time across the Globe, they faced, in the new high-latitude territories, an extremely different sunlight radiation, in both intensity and seasonality, and a significantly colder climate. In high latitude locations, during wintertime, the sun's rays reach the earth at a more oblique angle, taking therefore a longer path through the atmosphere, and are consequently less intense; indeed nearly no UVB (the adequate wavelength to produce vitamin D in the skin) is available at this time of the year at latitudes above 40°. It is thus believed that lightly pigmented skin evolved through time in order to adapt to the effects of the high latitude environment and optimize vitamin D production (1–3).

Vitamin D is vital to bone health and prolonged severe deficiency can lead to rickets in children and osteomalacia/osteoporosis in adults. Vitamin D is an exceptional nutrient in that its main source is exposure of the skin to UV rays, whilst it can also be ingested through diet. Accordingly, it has been generally assumed that populations in sunny (low-latitude) countries are ensured sufficient vitamin D levels to, at the minimum, prevent detrimental effects to bone health throughout the year.
However, over the past two decades, there has been mounting scientific and clinical evidence that vitamin D inadequacy, defined as 25-hydroxyvitamin D (25(OH)D) concentrations below 75 nmol/l, is a major public health issue not only across all age and ethnic groups, but also across different latitudes around the world. Whilst this has led to an increasing interest in vitamin D amongst the scientific community, governmental advisory bodies, the food and supplement industries, and more notably the general public, recommendations for optimal vitamin D status (25(OH)D concentrations above 75 nmol/l) as well as dietary intakes and sunlight exposure remain extremely controversial and are still much debated.

The current challenge in reaching a consensus on recommendations for vitamin D is mostly due to the lack of robust and comparable data from randomized controlled trials specifically designed to understand: A) the functional effects of low levels of vitamin D on bone health and non-skeletal health outcomes throughout the life cycle; B) the real effects of sunlight exposure on vitamin D concentrations according to local environment and individual behaviour; C) the actual contribution of dietary intake, from both food and supplements, to maintain adequate vitamin D status; and D) the differences in factors influencing vitamin D status, particularly between different ethnic groups. In addition, the variability in assessment methods and the influence of baseline levels thwarts direct comparisons between studies conducted in different locations and population groups.

Better understanding of the true unique contributions, as well as the interaction, of vitamin D dietary intake (from food and supplements) and sunlight exposure, along with influential factors, on serum vitamin D concentrations and consequent clinical outcomes, will greatly contribute to the determination of meaningful and context-specific recommendations for different populations. Subsequently, such knowledge will be key in determining public health strategies and policies for an efficient prevention and treatment of vitamin D inadequacy.
1.2 Vitamin D physiology and functions

Vitamin D is a misnomer as it is not actually a vital amine, but a pro-hormone, required throughout life. Several tissues and cells in the human body have been found to be potentially capable of converting 25(OH)D to the active form, 1,25-dihydroxyvitamin D$_3$ (1α,25(OH)$_2$D$_3$), and most of them actually have a vitamin D receptor.

The major physiologic function of vitamin D and its metabolites is to maintain calcium homeostasis for metabolic functioning, signal transduction and neuromuscular activity. The biologically active metabolite 1α,25(OH)$_2$D$_3$ is involved in bone formation as well as bone maturation (4–6). Along with parathyroid hormone (PTH), it regulates calcium and phosphorous metabolism and enhances the absorption of calcium in the gut and reabsorption of filtered calcium in the kidney. 1α,25(OH)$_2$D$_3$ is the only known hormone to induce the proteins involved in active intestinal calcium absorption (1,7).

When calcium concentrations decrease below normal physiologic levels, calcium-sensing proteins stimulate the secretion of PTH and the expression of PTH gene in order to restore calcium homeostasis. Consequently, the active 1α,25(OH)$_2$D$_3$ hormone stimulates intestinal calcium absorption or along with PTH, in the case of higher concentrations of the latter, increases mobilization of calcium from the bone and renal calcium reabsorption (4,7,8). If calcium concentrations exceed normal physiological concentrations, C-cells in the thyroid gland release calcitonin to supress calcium mobilization from bone. Circulating 1α,25(OH)$_2$D$_3$ then supresses parathyroid gland activity directly, decreasing PTH levels (Figure 1.1) (7)

With low vitamin D concentrations, calcium and phosphorus absorption in the small intestine are reduced to 10-15% and 60%, respectively. In contrast, with higher vitamin D concentrations, intestinal absorption can increase up to 30-40% for calcium and 80% for phosphorus (9).
Although data is still largely observational, recent evidence has also emerged proposing Vitamin D may have an important role in the pathophysiology of conditions as diverse as inflammatory and heart diseases, type I and II diabetes, various types of cancer and multiple sclerosis (9–11). The biochemical and functional outcomes of vitamin D status on skeletal health are further discussed in Chapter 5.

1.3 Vitamin D synthesis

Vitamin D is the generic term for two different molecules, ergocalciferol (vitamin D$_2$) and cholecalciferol (vitamin D$_3$) (Figure 1.2). Ergocalciferol is derived from UVB radiation on ergosterol, which is largely distributed in plants and fungi, whereas cholecalciferol is formed from the action of UVB rays in the skin and therefore present as well in animal origin foods (1,7). For the purpose of this thesis, vitamin D without a subscript represents either vitamin D$_2$ and/or D$_3$, unless otherwise specified.
Ergocalciferol (Vitamin D$_2$)

Cholecalciferol (Vitamin D$_3$)

**Figure 1.2** Structure of Vitamin D$_2$ and D$_3$, public domain from PubChem (PubChem Compound Database) (12)

Casual exposure of the skin to the UVB radiation portion of sunlight (wavelength between 290-315 nm) converts the molecule 7-dehydrocholesterol, naturally present in the epidermis, to pre-vitamin D$_3$. Pre-vitamin D$_3$ is thermodynamically unstable and thus, is quickly metabolized to become vitamin D$_3$ through a thermal isomerization. Vitamin D$_3$ then binds to vitamin D binding protein (DBP) in the bloodstream and is transported to the liver, along with vitamin D from dietary intake (food and supplements) (1,7,13).

In the liver, these molecules undergo a first hydroxylation by the cytochrome P450 enzyme CYP2R1 (25-hydroxylase) to produce the major circulating form, 25(OH)D. The molecule 25(OH)D undergoes a further hydroxylation in the kidneys, by enzyme CYP27B1 (1α-hydroxylase), resulting in the active form 1α,25(OH)$_2$D$_3$, which is the main biological active metabolite (1,7) (**Figure 1.2**).
Up to 90% of vitamin D in most individuals can come from the exposure of the skin to sunlight (1,2,14). However, human individual UV radiation from exposure to the sun depends mainly on sunlight availability (i.e. latitude and climate) but also the intensity and duration of direct exposure. Moreover the photochemical production of vitamin D depends on the amount of UVB rays actually reaching the skin and the amount of 7-dehydrocholesterol available in the epidermis (15–17). Hence, vitamin D synthesis is essentially dependent on a series of individual factors such as skin pigmentation, lifestyle, behaviour, age and adiposity.

1.4 Vitamin D from sunlight

Frequent casual exposure to sunlight has been long considered the most important source of vitamin D (2,18). Yet, cutaneous synthesis of vitamin D is mainly dependent on UVB rays reaching a 7-dehydrocholesterol molecule in the epidermis, which is influenced by the solar zenith angle and therefore latitude, local atmospheric conditions, season and time of the day (15,19,20).
The UV radiation level is a function of the position of the sun in the sky – the higher the sun in the sky, the higher the UV radiation level – varying with time of day and time of the year (season). The earth's tilted position with respect to its orbit around the sun, along with its yearly revolution and inherent daily rotation, determines the distribution of solar radiation over its surface. The solar zenith angle decreases with proximity to the equator, reducing the path length of sunlight through the atmosphere, and consequently increasing the effective level of UV radiation. Moreover, atmospheric conditions, such as cloud, ozone and humidity absorb much of the UV rays in sunlight before it reaches the surface (1,4,5).

A simple measure of the UV radiation level at the Earth’s surface is expressed as a Solar/Erythemal UV Index, a system developed by the World Health Organization (WHO) to foster public awareness about the health effects of exposure to UV radiation (21). As UV radiation levels vary throughout the day so does the index value. A UV index report emphasises the maximum UV radiation level on a given day, specifically during the 4 hours period around local solar noon (21). With higher index values the potential for damage from exposure to sun radiation to the skin and eye increases and less time is required for the detrimental effect to occur (Table 1.1).

<table>
<thead>
<tr>
<th>Exposure Category</th>
<th>UV Index Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Moderate</td>
<td>3 - 5</td>
</tr>
<tr>
<td>High</td>
<td>6 - 7</td>
</tr>
<tr>
<td>Very high</td>
<td>8 - 10</td>
</tr>
<tr>
<td>Extreme</td>
<td>11+</td>
</tr>
</tbody>
</table>

Based on the UV index categories, the WHO report suggests that within a UV index of 1-2 it is safe to stay outside; between 3-7 protection is required (shade, sunscreen or hats) especially during midday hours; and above 8 extra protection is needed and being outside during midday hours must be avoided (21).
In high latitude countries, there is no UVB radiation from the end of October to the end of March \((4,18,22,23)\). In contrast, in low latitude countries there is an abundant radiation of this wavelength throughout all year \((15,24)\). **Figure 1.4** illustrates the evident difference between high latitude countries and low latitude countries in sunlight radiation availability, during summer and winter seasons.

![Erythemal UV index -- climatology](image)

Winter at the Northern Hemisphere and summer in the Southern Hemisphere.
Summer at the North Hemisphere and winter in the Southern Hemisphere.

Solar noon UV Index average for 1996-2002, based on GOME spectrometer data from ESA's ERS-2 satellite, as published by KNMI (Royal Netherlands Meteorological Institute).

Figure 1.4 UV index at local solar noon across the globe (This work has been released into the public domain by its author, KNMI).

Due to the clear dependence on latitude gradient for adequate UV radiation for the production of vitamin D in the skin, it has been generally assumed that the most important determinant for optimal vitamin D levels is the geographical location where the individual lives. However, although the ambient and local UV radiation levels are objectively measured and defined, assessing the actual individual exposure of the skin is still a challenge and depends on many factors, which will be further discussed along this chapter.
A commonly used measurement for individual exposure is the standard erythemal dose (SED), a fixed physical measure used to quantify UV radiation levels based on erythema risk and is equivalent to 100 J/m². For instance, for a white-skinned adult, exposure of 6% of the body to sunlight, enough to result in slight pinkness to the skin 24 hours after exposure (1 minimal erythemal dose), could correspond to taking between 15 μg (600 IU) to 25 μg (1000 IU) of vitamin D orally (2,9,25). The minimal erythemal dose (MED) refers to the minimal radiation that will cause sunburn or redness to the skin, and thus will vary individually depending on skin type, time of year, behaviour and age.

1.4.1 The paradox of vitamin D deficiency versus skin cancer

In contrast with the benefits of casual sensible exposure to sunlight, prolonged exposure of the skin to intense solar UV radiation can lead to both acute and chronic health outcomes, including inflammatory effects, for the skin, eyes and immune system. In the long term the acute effects of sunburn and tanning from excessive exposure to sunlight can provoke further degenerative alterations in cells, and consequent premature skin ageing or development of cancerous cells (26).

The UVB action spectrum for pre-vitamin D formation in the skin overlaps considerably with the spectrum for detrimental effects from sunburn (erythema); thus sun avoidance to reduce the negative consequences from sunburn is likely to lead to a concomitant reduction in vitamin D synthesis (27). Figure1.5 exemplify these paradoxical overlapping effects by showing the official action spectra for human erythema from the Commission Internationale de L’Eclairage (CIE) action spectra for pre-vitamin D synthesis (27).
Figure 1.5 Overlap of actions spectra for human erythema and the synthesis of pre-vitamin D. Reproduced with permission from Springbett, Buglass and Young, 2010 (27)

1.4.2 Sunlight exposure recommendations

Few studies to this date have been specifically designed to examine the actual typical individual exposure to sunlight in high latitude dwelling populations over the different seasons, and even fewer in low latitudes. Due to the complexity of guidance on sunlight exposure considering both vitamin D adequacy and risk of skin cancer, it is evident that recommendations must be latitude-specific.

A recent study has shown that a white-skinned adult in the United Kingdom (latitude 51°N), spending 9-13 minutes outdoors at noon during spring/summer months in season-appropriate clothing would be able to maintain vitamin D concentrations at or above 25 nmol/l throughout the following winter (28). In the United States (latitude 37°N), it is generally recommended that exposure of arms and legs for 5 to 30 minutes between the hours of 10 a.m. and 3 p.m. twice a week would be enough for adequate levels, but taking into consideration time of day, season, latitude and skin pigmentation (9).
In contrast, for an adult with moderately fair skin pigmentation in Australia (latitude 25°S) and New Zealand (latitude 40°S), it is recommended that unprotected exposure to sunlight for 5-10 minutes, mid morning or mid afternoon on most days of summer, and 7-30 minutes (depending on latitude), midday on most days of winter, is sufficient to maintain adequate vitamin D levels for bone health purposes. For darker skin individuals, recommendations range from 15-60 minutes, most days of summer, and 20 min – 3 hours (depending on latitude), most days of winter (29).

In Brazil (latitude 14°S), there is no official guidance for sunlight exposure for the purpose of maintaining adequate vitamin D status. The Brazilian Society of Dermatology has very recently published a statement advising caution towards sunlight exposure due to the risks of skin cancer, especially in fair skinned individuals (30). This document recommended that exposure to sunlight in Brazil should be minimized or avoided between 10am and 3pm, and recommended the use of protective clothing and hats as well as the frequent use of sunscreen on uncovered body areas when out in the sun, at anytime of the day (30).

A guidance report from FAO/WHO recommends that the most efficient physiological approach to acquiring vitamin D for populations at the equatorial latitude range (42°N - 42°S) is through endogenous synthesis via sunlight exposure. The report specifically suggests that daily exposure of arms and face, without sunscreen, for approximately 30 minutes would be enough to maintain adequate vitamin D levels. Nonetheless, it also recognizes the negative influence of several environmental and individual factors (such as latitude and season, ageing, skin pigmentation, clothing and sunscreen use) on the production of vitamin D in the skin. Finally, the report recommendations for future research includes the need to better understand the relationship between latitude, sun exposure, and synthesis of vitamin D (31).
1.4.3 Factors affecting individual UV radiation levels and consequent cutaneous synthesis of vitamin D₃

1.4.3.1 Physiological factors

- Skin pigmentation

Skin pigmentation can greatly reduce the UV-mediated synthesis of vitamin D. Cutaneous melanin pigment in human skin naturally competes for and absorbs the UVB photons responsible for the photolysis of 7-dihydrocholesterol to pre-vitamin D₃. Therefore individuals with higher melanin (i.e. dark skinned) content in their skin require more UV light exposure to synthesize the same amount of vitamin D₃ as individuals with less melanin (i.e. fair skinned) (16,22,32).

In fact, observational studies consistently demonstrate that individuals with lighter skin have higher serum 25(OH)D concentrations than those with darker skin pigmentation (32,33). This difference was well observed in a study conducted in the United State, where white adults raised 25(OH)D concentrations more than 30 fold when compared to black adults exposed to the same amount of UVB radiation in a tanning bed (34). However, when the black adults were exposed to 5 times more UVB radiation, their blood level increased by about 15-fold (34).

- Aging

With aging, from 20 years of age onwards, the concentration of 7-dehydrocholesterol in the epidermis decreases linearly over the lifespan and thereby the capacity of the skin to produce vitamin D decreases and can be reduced by approximately 75% by 70 years of age (25,35). In a study with younger (<60 years) and older adults (60 – 80 years) exposed to a whole-body artificial UV radiation, mean 25(OH)D concentrations increased to a maximum of 78.1 nmol/l compared to 20.8 nmol/l (36).
- **Obesity**

A significant negative association between adiposity and low 25OHD in humans has been suggested in recent research. Reasonable explanations for this include sun exposure avoidance and limited mobility in overweight people, clothing habits, volumetric dilution in a larger body volume and decrease in bioavailability of vitamin D circulating as a consequence of enhanced uptake by adipose tissue (37–39).

In the Framingham Heart Study cohort (n=3,890), vitamin D status was found to be strongly associated with variation in subcutaneous and especially visceral adiposity, showing that higher adiposity volumes were correlated with lower 25(OH)D concentrations across different categories of Body Mass Index (BMI), including in lean individuals (BMI <25 kg/m²) (40).

- **Genetic factors**

Inter-individual variability in vitamin D status could be reasonably explained by differences in the metabolism of vitamin D. The VDR gene plays an important role in Vitamin D metabolism and polymorphisms in this gene can potentially affect Vitamin D gene expression, meaning that genetic variation could explain the considerable differences in vitamin D levels among population independently of latitude and sunlight exposure (41–43). The heritability of vitamin D status has been estimated to be 43% in a sample of 1068 twin pairs (primarily female mean age 45 years) and 28.8% in a cross-sectional study of 1762 participants of the Framingham Offspring Study (919 women; mean age 59 years) (43,44).

1.4.3.2 **Limited sun exposure**

- **Indoor environment**

Limited time outdoors can significantly reduce the amount of UV radiation received by the individual and therefore limit the cutaneous production of vitamin D. Modern society structures have developed in a way that in most countries nowadays, the urban industrialized setting
involves significantly higher amounts of time spent indoors rather than outdoors (3,45). That setting accounts for office-based working hours (usually during daylight hours) and a sedentary lifestyle, that besides reducing the amount of time outdoors in comparisons to active peers, also includes a preference for private or public automotive vehicles rather than cycling or walking for routine transit. Moreover, even for those who are physically active, it is not uncommon for sports training and physical activity to take place indoors. In fact, individuals training outdoors have been reported to present higher 25(OH)D concentrations compared with those training indoor have (1,46,47).

Several studies have also shown that older, hospitalised or institutionalised populations are at a greater risk of having vitamin D inadequate levels due to the reduced or almost no time spent outdoors (48–51)

- **Sun avoidance (shade, sunscreen) and clothing cover**

Over the past decades, evidence of the associations between sunlight exposure and skin cancer have increased, especially in sunny, low latitude countries (26,52). This increasing awareness of higher risk of skin cancer related to direct sun exposure and might be influencing skin synthesis of vitamin D adversely. Regular use of sunscreen, direct sun avoidance and “covering up” are largely advised in sunny countries, particularly in countries with high incidence of skin cancer like Australia and Brazil (30,53,54).

Sunscreen is the most common and practical approach for skin photo-protection. It is designed to absorb, reflect or scatter UVB radiation reaching the skin and therefore attenuates the solar radiation reaching the cells in the skin. Topical correct application of sunscreen, of a sun protection factor as low as 8, can significantly reduce the production of endogenous vitamin D (27,55). However, it has been recently argued that, although regular topical use has been proven to be effective against detrimental effects to the skin exposed areas, individuals are not likely to use sunscreen products appropriately enough to prevent vitamin D production (56,57).

Clothing habits due to cultural or religious preferences are also an important influencing factor that may contribute to a significant reduction in the synthesis of vitamin D (15,58). Individuals
that wear clothing that covers most of the body have a greater risk of having vitamin D deficiency as the area exposed to sunlight is significantly reduced.

- **Air pollution**

  Industrialized cities are likely to have considerable air pollution containing elevated amounts of ozone, which efficiently absorbs UVB radiation (more specifically solar radiation below 290 nm). Less availability of UVB radiation reaching the skin reduces the production of endogenous vitamin D (15,59,60). Some studies suggest an association between air pollution levels and 25(OH)D status, where higher 25(OH)D concentrations observed in populations at less polluted areas of a city (59,61,62).

- **Cloud, Ozone and Altitude**

  Atmospheric conditions, such as cloud, ozone and humidity can cause absorption or deflection of much of the UV rays in sunlight before it reaches the earth’s surface (5,63). Under cloudless skies UV radiation levels are significantly higher although even with cloud cover, UV radiation levels can be high depending on latitude and time of the day. Conversely, UV radiation can be reduced by 50% in the shadow (15,60).

  The thinner atmosphere at higher altitudes results in less UV radiation absorption (10% to 12% increase in radiation with every 1000 metres increase in altitude) (17,64). Few studies have directly assessed varying altitude and concurrent vitamin D production but one study, conducted at 27° N using ampoules of 7-dehydrocholesterol placed at varying altitudes between (ranging from 169m to 5350m above sea level), showed a 4 times greater vitamin D production at higher altitudes and 2 times greater at intermediate altitudes (Kathmandu - 1400m compared with Agra – 169m) (65).
### Season

In summer, due to the sun’s higher position in the sky, its rays hit the Earth more directly, and therefore less radiation is spread out. In contrast, during winter the sun appears low in the sky, spreading its rays out over a much wider area, becoming less effective (15,17,60). Accordingly, a seasonal cycling of serum 25(OH)D concentrations has been shown in countries at mid-high (32,66,67) as well as low tropical latitudes (54,68,69).

A recent two-centre cohort study with 518 postmenopausal women (age 55-70 years) assessed serum 25(OH)D at fixed three-monthly intervals from summer 2006 and observed significant local, seasonal and ethnic differences in vitamin D status for postmenopausal women at high latitudes. At 57° N (Aberdeen, Scotland, UK), Caucasian women had lower 25(OH)D (p < 0.001) compared to 51° N (Surrey, South of England, UK). Median (interquartile range) in nmol/l were 43.0 (20.9) and 62.5 (26.6) in summer and 28.3 (18.9) and 39.9 (24.0) in winter, at 57° N and at 51° N, respectively. For Asian women at 51° N, median 25(OH)D was 24 (15.8) nmol/l in summer and 16.9 (15.9) nmol/l in winter (19).

In lower latitudes, the same seasonal variation is observed, and although the minimal sunlight radiation during winter-time is still of high enough levels for adequate vitamin D production, the seasonal cycle in 25(OH)D concentrations has also been reported in such locations (54,68,69).

### 1.5 Dietary Vitamin D

#### 1.5.1 Sources

The two main forms of vitamin D are naturally present in a few foods, although in small quantities, with ergocalciferol (vitamin D₂) from plant and/or fungal sources, such as mushrooms and cholecalciferol (D₃) from animal origin foods such as oily fish, eggs and liver. It is important to appreciate that the nutrient content of foods can vary significantly between different countries as well as habitual intake of specific foods. Table 1.2 presents the vitamin D content of selected foods.
Table 1.2 Dietary Vitamin D Content (μg/100g) of Selected Foods

<table>
<thead>
<tr>
<th>Food Source</th>
<th>Vitamin D (μg/100g)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs (raw)</td>
<td>3.2</td>
</tr>
<tr>
<td>Meats</td>
<td></td>
</tr>
<tr>
<td>Beef, steak, grilled</td>
<td>0.8</td>
</tr>
<tr>
<td>Pork, grilled</td>
<td>0.9</td>
</tr>
<tr>
<td>Chicken, grilled</td>
<td>0.3</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
</tr>
<tr>
<td>Salmon, wild, raw</td>
<td>8.6</td>
</tr>
<tr>
<td>Salmon, farmed, raw</td>
<td>4.7</td>
</tr>
<tr>
<td>Mackerel, raw</td>
<td>8.0</td>
</tr>
<tr>
<td>Sardines, canned</td>
<td>3.6</td>
</tr>
<tr>
<td>Mushrooms (wild)</td>
<td>13 - 30</td>
</tr>
</tbody>
</table>

¹ Values taken from McCance and Widdowson’s The Composition of Food (70)

1.5.2 Dietary Recommendations

The Institute of Medicine (IOM) and the Endocrine Society in the US and the Brazilian Society of Endocrinology and Metabology (SBEM) in Brazil all recommend to the general population a daily dietary intake of 15 μg of vitamin D for individuals between 1 and 70 years and 20 μg for those > 70 years. However, groups considered to be at high risk may need higher intakes (Table 1.2) (71–73).

Previously in the UK, an Reference Nutrient Intake (RNI) for vitamin D was set only for population groups deemed to be at high risk of deficiency, assuming that for most people the amount of vitamin D synthesized in the skin by exposure to sunlight was sufficient to achieve serum 25OHD concentrations above ≥ 25 nmol/l throughout the year. However, increasing new evidence proved this not to be the case and a reviewed RNI for vitamin D of 10 μg/d has been recently proposed for the UK population aged 4 years and over, including individuals from minority ethnic groups with darker skin (Table 1.3) (74).
### Table 1.3 Vitamin D Dietary recommendations from different Advisory bodies: IOM, SBEM, Endocrine Society and SACN (71-74)

<table>
<thead>
<tr>
<th>Age groups</th>
<th>IOM / SBEM / Endocrine Society</th>
<th>SACN Whole population (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>General population (µg)</td>
<td>Population at risk (µg)</td>
</tr>
<tr>
<td>0 – 12 months</td>
<td>10</td>
<td>10 – 25</td>
</tr>
<tr>
<td>1 – 4 years</td>
<td>10</td>
<td>15 – 25</td>
</tr>
<tr>
<td>4 – 8 years</td>
<td>10</td>
<td>15 – 25</td>
</tr>
<tr>
<td>9 – 18 years</td>
<td>15</td>
<td>15 – 25</td>
</tr>
<tr>
<td>19 – 70 years</td>
<td>15</td>
<td>37.5 – 50</td>
</tr>
<tr>
<td>&gt; 70 years</td>
<td>20</td>
<td>37.5 – 50</td>
</tr>
<tr>
<td>Pregnant women 14 – 18 years</td>
<td>15</td>
<td>15 – 25</td>
</tr>
<tr>
<td>Pregnant women &gt; 18 years</td>
<td>15</td>
<td>37.5 – 50</td>
</tr>
<tr>
<td>Lactating 14 – 18 years</td>
<td>15</td>
<td>15 – 25</td>
</tr>
<tr>
<td>Lactating &gt; 18 years</td>
<td>15</td>
<td>37.5 – 50</td>
</tr>
</tbody>
</table>

1 IOM: Institute of Medicine (US, 2011); SBEM: Brazilian Society of Endocrinology and Metabolism (Brazil, 2014); Endocrine Society (US, 2011); SACN: Scientific Advisory Committee on Nutrition (UK, 2016).

2 A separate RNI is not required for at-risk groups; ^a "Safe Intake"

### 1.5.3 Supplementation and fortification

Due to the limited contribution of foods/diet to vitamin D status, vitamin D supplementation and/or food fortification have been increasingly proposed worldwide as an effective strategy to tackle vitamin D low levels. In fact, it is estimated that with a western diet, an individual is not likely to exceed 5 µg/day through diet alone (4).

Vitamin D supplementation has been shown to improve serum 25(OH)D concentrations and subsequently improve the absorption of calcium and suppress PTH secretion (75–77). Vitamin D supplementation has gained an increasing popularity over the past decades, with widespread claims of preventive or therapeutic effects to a diverse range of health outcomes (10,78). A national survey from 2011-2012 in the United States reported that the prevalence of vitamin D supplementation in the population increased to 19% compared to 5.1% in 1999-2000 (79).
However, despite the increasing popularity of vitamin D supplements, the efficacy and safety is still much debated amongst scientific literature and more robust data from randomized control trials are needed, especially in regards to different health outcomes and different population groups. Moreover, vitamin D supplementation dose and regimen chosen should take into consideration the level of deficiency and the target to be achieved.

Some countries already have evidence of vitamin D fortified foods making an impact in improving vitamin D status in their populations as a whole. A systematic review and meta-analysis of randomised control trials assessing the impact of consuming vitamin D fortified foods on 25(OH)D status found that 11 μg (440 IU) per day resulted in an increase in 25(OH)D concentration of 19.4 nmol/l (80).

It is possible to find commercially fortified foods (milk and dairy products, orange juice, bread and cereals), with amounts per portion varying by country (81). In the United Kingdom, for instance, although fortification is no longer mandatory, most margarines and fat spreads are presently still fortified with vitamin D on a voluntary basis (usual levels ranging from 2.5 μg to 7.5 μg per 100 g) (74). Other foods, such as breakfast cereals and dried or evaporated milks, may also be fortified on a voluntary basis, as well as infant formulas. In the United States, milk and milk substitutes are fortified, also on a voluntary basis around 1 μg per 100 g. In Canada fortification of milk and margarine is mandatory (81) at levels of 1 μg/100 g and 13.5 μg/100 g respectively. In Australia, margarine and milk and milk products are currently fortified with vitamin D (29). In Brazil no official recommendations for fortification of foods with vitamin D have been established, although vitamin D fortified dairy products are available (usual levels ranging from 0.5 μg to 6 μg per 100 g) (82).

Both vitamin D₂ and D₃ can be commercially manufactured and added to supplements and fortified foods. Nevertheless, daily vitamin D₃ has been recently shown to be more effective than vitamin D₂ in increasing serum 25(OH)D in the wintertime (83,84) and, consequently, Vitamin D₃ supplementation/fortification has been currently recommended as better option to achieve optimal vitamin D status within the general population.
1.4 Vitamin D Status

1.4.1 Measurement of serum 25(OH)D concentrations

The metabolite 25(OH)D is the major circulating form of vitamin D, with a half-life of approximately 2 to 3 weeks and excellent stability. It reflects Vitamin D from both dietary intake and photo-chemically produced in the skin. Therefore it is universally accepted as the best indicator of vitamin D status (71,74). The active form 1,25(OH)₂D is chemically unstable and has a half-life of a few hours, and therefore not recommend as an indicator of vitamin D status (1,4).

Circulating 25(OH)D can be measured by several methods, including immunoassays, protein binding assays, high performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). The significant variability in results due to the availability of many differing methods poses a major challenge not only to the interpretation and to the comparison of different data sets, but particularly to the development of international evidence based recommendations. There is a general consensus that 25(OH)D assays should detect both metabolites 25(OH)D₂ and 25(OH)D₃. HPLC and LC-MS/MS methods detect both D₂ and D₃ metabolites and, thus, are currently considered the gold standard for 25(OH)D measurement. (85).

Moreover, evidence has shown significant differences even in the same laboratory as inter-assay variations in standard procedures may lead to very different results. The Vitamin D External Quality Assessment Scheme (DEQAS), established in 1989, has significantly helped to improve assay performance and development via external control of accuracy. In 2010, the NIH Office of Dietary Supplements (ODS) together with the CDC National Centre for Environmental Health (NCEH), National Institute of Standards and Technology (NIST) and Ghent University, proposed the Vitamin D Standardization Program (VDSP). The main objective of VDSP is to increase the comparability of data from different national surveys around the world (86–88).

Although protocols for standardization of 25(OH)D data are a relevant facilitator of cross-population comparisons, there are a few other critical aspects that should also be taken into
consideration in assessing vitamin D status. Individual variation might be driven by the time of the year the samples are collected, whilst results may over or underestimate ‘true’ values due to confounders such as holidays to a sunnier/colder location during the sampling period, vitamin D supplement intake, medication or medical treatment likely to affect vitamin D metabolism or use of sunbeds, etc. (20,24).

### 1.4.2 Optimal vitamin D status

The most common criteria used for determining the optimal serum 25(OH)D concentration for bone health in adults include the suppression of parathyroid hormone secretion, higher bone mineral density, reduced rates of bone loss and decreases in fractures and falls (8,9,89).

There is still much debate and controversy about which levels of circulating 25(OH)D should be considered as deficient, insufficient and sufficient and consequently there is currently no consensus on a definition of optimal vitamin D status (Figure 1.6). There is though, a general agreement that circulating 25(OH)D concentrations of populations should not fall below 25 nmol/l in order to preserve bone health (71,74,90).

![Deficiency and adequacy thresholds for circulating 25(OH)D proposed by different international advisory agencies (in nmol/l) (25,71,74,91).](image)

**Figure 1.6** Deficiency and adequacy thresholds for circulating 25(OH)D proposed by different international advisory agencies (in nmol/l) (25,71,74,91).
The UK Scientific Advisory Committee on Nutrition (SACN) defines vitamin D deficiency as 25(OH)D concentrations below 25 nmol/l, the IOM in the US defines insufficiency as 25(OH)D concentrations below 50 nmol/l and the US Endocrine Society proposes 75 nmol/l as the minimum level required to prevent detrimental effects to health (25,71,74).

The Brazilian Society of Endocrinology and Metabology very recently proposed a new consensus on vitamin D status recommending that 25(OH)D levels should be above 50 nmol/l for the general population. Those considered at a higher risk for vitamin D deficiency should aim for serum concentrations between 75 and 150 nmol/l, and an upper tolerable concentration of up to 250 nmol/l (91).

1.5 Toxicity and safety

Remarkably, vitamin D metabolism is tightly regulated to counteract excess production of vitamin D from sunlight exposure, which could lead to severe toxicity and potentially hypercalcemia, causing renal failure and cardiac arrest (1,4,92,93). In the case of an excessive exposure to sunlight, pre-vitamin D₃ and vitamin D₃ are degraded into biologically inactive photoproducts (2,7,11). For dietary intake, the Food and Nutrition Board Dietary Reference Intakes (DRIs) proposes 2,500 IU of vitamin D₃ daily as the safe Tolerable Upper Intake Level (UL) (94).

There has been no adverse effects or toxicity cases reported in trials with adults receiving up to 10,000 IU of vitamin D₃ daily (92,95,96). Moreover, the concentration that would be associated with hypercalcemia is estimated to be as high as 600 nmol/l and at doses above 25,000 IU per day (625 mcg) (92,93,95,96).

1.6 Vitamin D status and intake in populations worldwide

Over the past three decades, increasing prevalence of inadequate vitamin D status has been reported in different populations worldwide (11,23,97–99). The majority of the data comes from studies and national surveys in high latitudes countries, which is not surprising due to the
known challenges of maintaining adequate vitamin D levels in these locations, especially during winter. More recently, evidence has emerged indicating alarming prevalence of low vitamin D concentrations in low latitude countries, despite the typical sunlight abundance (100–103). A review from the International Osteoporosis Foundation described the vitamin D status in the general population of 46 countries worldwide based on 200 publications from 1990 to 2011, presenting the data on a global map (Figure 1.6) (104).

![Figure 1.6](image1.6.png)

**Figure 1.6** ‘Snapshot’ of 25(OH)D levels around the globe, as identified in publications since the year 1990, by the International Osteoporosis Foundation. Reproduced with permission from (104)

A recent study applying VDSP protocols to 14 studies, combined with 4 previously standardized studies, from representative European populations, estimated that 40% and 13% of 55,844 individuals had average year-round 25(OH)D concentrations below 50 and 30 nmol/l respectively. Remarkably, dark-skinned ethnic groups were estimated to have higher prevalence of levels below 75 nmol/l, from 3 to up to 71 fold, compared to white individuals (99).

A multinational study conducted in 2004/2005 that included 18 countries ranging from latitude 64°N to 38°S with 2606 participants observed that low 25(OH)D concentrations were common amongst postmenopausal women with osteoporosis, with 64% of this sample having levels
below 75 nmol/l. Mean 25(OH)D concentration was 67 (SE 0.75) nmol/l and values ranged from 15 to 607 nmol/l, with regional mean concentrations lowest in the Middle East (51 nmol/l, SE 1.25) and highest in Latin America (74 nmol/l, SE 1.5) (48).

In the United States, data from the National Health and Nutrition Examination Survey (NHANES) population reported that the number of individuals with 25(OH)D concentrations below 75 nmol/l almost doubled from 1994 to 2004 reaching nearly 75% of the American population. Within pigmented populations (Black, Hispanic and Asians) the prevalence of levels below 75 nmol/l was more than 90% in this cohort (105).

In the UK, data from the National Diet and Nutrition Survey (NDNS) has provided evidence of an increased risk of vitamin D deficiency in all age/sex groups. Year-round, the proportion of children with a serum 25(OH)D concentration below 25 nmol/l ranged from 7.5% for children aged 1.5 to 3 years to 24.4% for girls aged 11 to 18 years and for adults ranged from 16.9% for men aged 65 years and over to 24.1% for women aged 65 years and over. The proportion of participants with a serum 25(OH)D concentration below 25 nmol/l was higher in the winter months (106). The recent SACN report stated that the mean intake of vitamin D (from all sources, including supplements) for the general British was 2-4 µg/d for ages 1.5-64 years of age and 5 µg/d for adults aged 65 years or over (74).

A longitudinal cohort study in South Australia (latitude 34°S) with 2413 participants, conducted between 2008 and 2010, observed an overall mean serum 25(OH)D of 69.2 nmol/l with 22.7% of the population having concentrations below 50 nmol/l (54). Another study in Australia, with 126 healthy free-living adults (aged 18-87 years) found a prevalence of 10.2% and 32.3% of individuals with serum 25(OH)D concentrations below 25 and 50 nmol/l, respectively, at the end of winter (14).

In Brazil, recent studies have shown a high prevalence of vitamin D insufficiency and inadequacy in different latitudes across the country. Studies conducted in several different cities in Brazil found a high prevalence of 25(OH)D concentrations below 50 nmol/l, with values as high as 42% in the city of São Paulo (latitude 23°S), 31.5% in Recife (latitude 8°S), 63.7% in Curitiba (latitude 25°S), the latter with adolescents (100, 107,108). Alongside this, several national studies have shown that the Brazilian usual food intake is not a relevant source of vitamin D (Maeda et al. 2014). The most recent report on dietary intakes in the Brazilian
population estimated that the mean vitamin D intake in adults over 19 years of age was 3.2 μg/d (128 IU) for men and 2.9 μg/d (116 IU) for women (109).

1.7 Thesis Rationale

Vitamin D production in the skin, via sunlight exposure, has been long considered the main determinant of adequate vitamin D status for the maintenance of calcium homeostasis and bone health. In higher latitudes during the winter months, as the distance from the equator increases, so too does the zenith angle of the sun. Consequently, the UV radiation reaching the earth’s surface is reduced, resulting in limited cutaneous production of vitamin D₃. Thus, the cutaneous synthesis of vitamin D is expected to be greater in low-latitude regions due to greater exposure to UVB radiation.

Over the past 15 years an increasing prevalence of low levels of serum vitamin D have been observed across a number of low latitude countries, contradicting the longstanding assumed location-dependence for the prevalence of vitamin D deficiency and insufficiency. Moreover, although still an important source when UVB radiation is inadequate, vitamin D dietary intake has been shown to be suboptimal in most countries across the world.

Therefore, a robust full characterization of both the relative and combined contribution of diet and sunlight to achieving optimal vitamin D status is still required. Furthermore, the response of individuals to vitamin D supplementation is known to be variable in all population groups, since several individual and environmental characteristics may affect the conversion of pre-vitamin D by sunlight, such as latitude and available UVB radiation throughout the year, skin pigmentation, age, individual behaviour, diet and genetic factors.

1.7.1 Identification of Knowledge Gaps

There is a clear need for consistent data to establish realistic and meaningful recommendations of vitamin D requirements, particularly for different ethnic/racial groups. Although the literature is unanimous on the major contribution of sunlight exposure to the production and
maintenance of adequate vitamin D levels, people in high latitude countries cannot rely on this source for half of the year while people in low latitude countries may not have the assumed ideal exposure to sunlight due to being consistently advised to protect against sun radiation or due to clothing cultural habits. Moreover, the dietary intake values recommended by most advisory bodies are currently not being achieved by the majority of populations worldwide due to a lack of foods naturally rich in vitamin D and a lack of mandatory fortification of common staples in most countries.

The public health recommendations and messages around adequate sunlight exposure and vitamin D intakes (whether by diet or supplements) are currently confusing for most people, and do not take into account important influential factors such ethnicity and skin pigmentation, cultural behaviour, latitude of residence and season. Additionally, a lack of consensus on desirable 25(OH)D concentrations worldwide means it is difficult to recommend a vitamin D intake required to achieve an optimal level, again further confounded by potential ethnic differences in metabolism between different population groups. Another barrier to achieving consensus on optimal levels and subsequent lifestyle recommendations is the impossibility of making direct comparisons between, and thus extrapolating study findings from, countries located in different latitudes, with different cultural habits, availability of vitamin D food sources, skin pigmentation spectrums and lifestyle.

Very few studies so far have been specifically designed to investigate the effect of vitamin D supplementation and spontaneous-individual sunlight exposure together on serum vitamin D levels in healthy adults. To our knowledge, to date, there are no studies that have conducted fundamentally comparable vitamin D supplementation randomized controlled trials (RCT) in significantly different latitudes, with identical methodologies and same sex and ethnicity populations, to investigate the real contribution of each vitamin D source and the real impact of latitude of residence on both serum 25(OH)D levels and the beneficial effects of vitamin D supplementation, without the confounding from ethnicity.

Therefore, the main objective of this thesis is to address this crucial knowledge gap by investigating the interaction and relative contribution of vitamin D supplementation and individual sunlight exposure in raising vitamin D levels above recognized minimal vitamin D status cut-off points, throughout winter months, in ethnically identical adult women living in opposite latitudes (Chapter 4).
To further contribute to the need for meaningful vitamin D intake recommendations, the secondary aims of this Thesis include:

1) Examine the baseline serum vitamin D concentrations and potential influential factors in the studied populations within each country in order to account for the latitude difference (Chapter 3);

2) Investigate if there is a threshold serum 25(OH)D concentration where a plateau in plasma PTH is evident, which could be useful in setting cut-off threshold for vitamin D adequacy in this population (Chapter 3);

3) Explore on the association between vitamin D and bone parameters to further contribute to the understanding the important role of vitamin D in bone health outcomes (Chapter 5).

Specific to the ethnicity targeted in this study, very few consistent population studies so far in Brazil have evaluated vitamin D status as well as vitamin D dietary intake. There is also a considerable lack of data in Brazilian populations regarding their response to vitamin D supplementation since, to our knowledge, to date no randomized controlled trials with vitamin D supplementation in healthy adult women have been conducted in Brazil. In addition to the general contribution to the field on the interaction of vitamin D supplementation and individual sunlight exposure, this thesis also aims to provide key data to inform the future and much needed specific dietary and sunlight exposure recommendations for vitamin D adequacy in Brazilian women.
The D-SOL Study: Overview and Methods

The D-SOL study (Interaction between vitamin D supplementation and Sunlight exposure in adult women living in Opposite Latitudes) aims to contribute high quality scientific evidence to prevent vitamin D deficiency and insufficiency in adult women, regardless of where they live, and to provide key data to contribute to dietary recommendations for vitamin D in Brazilian women specifically.

The D-SOL study investigates the relative contribution of daily individual exposure to sunlight and vitamin D₃ daily supplementation on serum 25(OH)D, by comparing directly, using the same methodology, same sex and ethnicity individuals living in a low latitude location in the Southern Hemisphere - where there is abundant sunlight exposure, and in a high latitude location in the Northern Hemisphere - where exposure to sunlight is limited.

2.1 Hypotheses

- **Hypothesis 1**: Dietary intakes of vitamin D are too low and sunlight exposure is insufficient for maintaining optimal vitamin D status throughout wintertime in Brazilian women, regardless of latitude of residence.
- **Hypothesis 2**: Vitamin D supplementation is effective in raising and maintaining adequate 25(OH)D concentrations throughout winter, regardless of latitude.
- **Hypothesis 3**: The response of Brazilian women to vitamin D supplementation is dependent on baseline 25OHD levels.
- **Hypothesis 4**: Circulating 25(OH)D and PTH concentrations are associated with bone health parameters.
2.2 Study design

This was a multicentre study that consisted of two parallel, double-blinded randomised placebo-controlled trials. Participants were randomised to receive a daily supplement of 15 μg (600 IU) vitamin D3 or placebo, for 12 weeks during wintertime (Figure 2.1).

The full study protocol can be found in Appendix II. The D-SOL study was registered at clinicaltrials.gov as NCT03318029.

Figure 2.1 Flowchart of study design
2.2.1 Sample size and power calculation

Using data from previously published research (100), a total of 80 adult women (at 90% power) would be required to demonstrate a response to vitamin D supplementation in the range of 20-25 nmol/l at $\alpha = 0.05$ (n = 32 in each intervention group). The study numbers of 40 subjects for the supplemented group and 40 for the placebo group includes a 25% drop-out rate factored in.

2.2.2 Ethical approval

The study was approved by the University of Surrey (UEC/2016/009/FHMS) and Federal University of Goiás Ethics Committees and by the Brazilian National Ethics Committee (CONEP) (CAAE 62149516.9.0000.5083, CEP-UFG nº2013222; CONPEP nº 1972029; respectively). Approval letters can be found in Appendix I.

The study was conducted in accordance with the Helsinki Declaration and written informed consent was obtained from all participants. The consent form content for each country varied slightly, in order to be in accordance with the specific requirements of each country’s Ethics Committees (Appendix II). For participants living in England, the original consent form approved by the Ethic’s committee was translated from English to Portuguese and offered to participants in the England trial that felt more comfortable reading in their native language. For participants in Brazil, only a Portuguese version of the Brazil trial informed consent was provided.

2.2.3 Study location

The study trials were conducted in two opposite latitudes: Surrey, Southern England (51°N) and Goiás, Mid-west Brazil (16°S). Southern England has a temperate climate with a summer mean temperature of 22°C and winter mean temperature of 6°C, whereas Mid-west Brazil has a typical tropical savannah climate, with a summer mean temperature of 26°C and winter mean temperature of 24°C. While all four seasons in Southern England are well defined, with
significant differences between autumn/winter and spring/summer months, Mid-west Brazil is limited to two dominant seasons throughout the year, dry and wet, with high temperatures almost all year round. More importantly for the purposes of this thesis, the UV index never exceeds 8 in the UK (peaking towards the end of June), and in clear contrast, the minimum in Brazil is 8 reaching up to 14 during summer months. Table 2.1 presents these climatological differences in more detail.

Table 2.1 Main differences in climatological factors between Southern England and Mid-west Brazil.

<table>
<thead>
<tr>
<th></th>
<th>Surrey, UK¹</th>
<th>Goiás, Brazil²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude</td>
<td>51° N</td>
<td>16° S</td>
</tr>
<tr>
<td>Thermal zone</td>
<td>Temperate</td>
<td>Tropical</td>
</tr>
<tr>
<td>Seasons</td>
<td>4 seasons well defined</td>
<td>Wet/dry</td>
</tr>
<tr>
<td>Day length during winter (in hours)</td>
<td>~ 8</td>
<td>~ 11</td>
</tr>
<tr>
<td>Day length during summer (in hours)</td>
<td>~ 16</td>
<td>~ 13</td>
</tr>
<tr>
<td>Temperature Summer a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum (in °C)</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Maximum (in °C)</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td>Temperature Winter a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum (in °C)</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Maximum (in °C)</td>
<td>8</td>
<td>31</td>
</tr>
<tr>
<td>UV index year-round</td>
<td>0 to 8</td>
<td>8 to 14</td>
</tr>
<tr>
<td>UVB availability during winter</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

¹ Met Office, UK  
² Instituto Nacional de Meteorologia, Brazil  
³ Average over past years  
⁴ Approximately
2.2.4 Recruitment

Participants in England were recruited by Marcela Mendes, through advertisement among local Brazilian societies/groups within Surrey and London (latitude 51°N). Information posters (Appendix II) were positioned around the University of Surrey and commercial centres (with a focus on Brazilian themed places), with permission of the owners.

In addition, Brazilian institutions in the UK, such as the Brazilian Embassy in London (Dr Juliana Bertazzo) and the Brazilian Researchers Association (ABEP-UK) agreed to circulate a recruitment letter to their contact list of Brazilians living in England. The letter was sent directly by the institutions (Appendix II).

Participants in Brazil were recruited from the general public within the city of Goiânia (latitude 16°S) by Marcela Mendes, with the assistance of undergraduate students Emanoelly Pires and Giovana Caixeta, through posters positioned around the Federal University of Goiás and commercial centres, with permission of the owner.

For both trials, social media online platforms such as Facebook and Instagram were used to publicise the study: the recruitment poster was inserted as a photo with the permission of the administrators of the pages.

2.2.5 Screening and inclusion/exclusion criteria

If participants wished to be screened for participation in the study, they received a Participant Information Sheet (Appendix II) and were checked against the study inclusion and exclusion criteria (Table 2.2) using the ‘Screening Questionnaire’ (Appendix II), administered by Marcela Mendes over the phone or self-reported via email. The inclusion and exclusion criteria for participant recruitment are detailed in Table 2.2 below.
Table 2.2 Inclusion and exclusion criteria for the D-SOL study trials

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Brazilian nationality (born in Brazil and having at least one parent born in Brazil)</td>
<td>• Currently receiving treatment for medical conditions that are likely to affect vitamin D metabolism (osteoporosis therapy, anti-estrogens treatment, antiepileptic drugs, breast-cancer treatment)</td>
</tr>
<tr>
<td>• Female</td>
<td>• Hypercalcaemia (&gt;2.5mmol/L) – assessed and excluded at baseline</td>
</tr>
<tr>
<td>• Aged 20-59 years</td>
<td>• Regular use of sun-beds</td>
</tr>
<tr>
<td></td>
<td>• Having a holiday trip one month prior to commencing the study, plans for a holiday trip for more than 4 weeks out of the country of residence within the study period or a trip abroad within the last month of intervention</td>
</tr>
<tr>
<td></td>
<td>• Use of supplements containing vitamin D (if the prospective participants agreed to stop Vitamin D supplementation to join the study, a wash-out period of 8 weeks prior to commencing the trial was accepted)</td>
</tr>
<tr>
<td></td>
<td>• Pregnant or planning a pregnancy during the study period</td>
</tr>
<tr>
<td></td>
<td>• Not post-menopausal</td>
</tr>
<tr>
<td></td>
<td>• Living in England for less than 2 months (for England trial only)</td>
</tr>
</tbody>
</table>

2.2.6 Randomisation and blinding

Enrolled participants were randomised stratified by age and BMI classification using a computer-generated block randomisation programme. Dr Sig Johesen, a statistician at the University of Surrey not involved in the study produced a consecutive list of study identification codes linked to an intervention group.

The 15 μg vitamin D supplement and matching placebo were identical in appearance and taste, and were provided by Viridian Supplements (United Kingdom). The vitamin D₃ supplements
contained cholecalciferol extracted from Lichen and therefore were suitable for vegans. Tablets were packed in identical, not identifiable, bottles labelled with the identification number. All study researchers and staff as well as participants remained blinded to the supplement dose throughout the duration of the study. A researcher from the University of Surrey not involved in the study was responsible for keeping the codes during the study.

### 2.2.7 Intervention

The first clinical trial was conducted in England (latitude 51°N) from October 2016 to March 2017 (autumn-winter) followed by the second trial in Brazil (latitude 16°S) from April 2017 to September 2017 (autumn-winter). Both trials were conducted during the respective wintertime for each country to ensure minimal confounding from range differences in UVB radiation seasonal intensity. Marcela Mendes personally conducted and supervised both trials, as principal investigator. The 12-week intervention period was chosen to allow sufficient time for 25(OH)D concentrations to rise (half-life of 25(OH)D is 3 weeks) and has been demonstrated to be an effective timeframe for supplementation of vitamin D in previous studies (110).

The participants selected were randomly allocated to receive a daily supplement containing zero (placebo) or 15 μg (600 IU) vitamin D. The supplement dose was chosen to enable the study to be relevant to the IOM RDA recommendations for vitamin D₃ daily intake (71).

At baseline, participants were given a bottle with a total count of 100 tablets with either placebo or 15 μg Vitamin D₃, enough for over 12 weeks supplementation. Bottles were labelled with participant identification code, D-SOL study logo and brief instructions with contact number of principal investigator. Participants were instructed to take the supplement at the same time every day and together with a meal.

Participants were instructed to maintain their habitual sunlight exposure and protective measures (if part of habitual routine) as well as usual dietary intake and physical activity during the duration of the study and report any significant changes to their habits or normal routine.
Compliance was monitored by compliance interviews and by counting the remaining tablets from returned bottles at the end of the study. Throughout the intervention period, participants were contacted via telephone or email weekly to check for adverse events and encourage compliance.

### 2.3 Data Collection

Marcela Mendes conducted and supervised the data collection in both countries in person, as principal investigator. Data collection was assisted by Saskia Wilson-Barnes, Dr Philippa Gibson and Hajrah Mukthar in England, and Sáskia Vaz, Amanda Oliveira and Marília Bohnen in Brazil.

Before starting the study procedures, participants were given a printed copy of the Participant Information Sheet, and given time to read the document and ask any questions regarding the study. If they wished to proceed in taking part in the study, informed consent was discussed and participants were asked to sign the consent form, and offered a copy to keep for themselves.

Participants visited the respective universities, University of Surrey in England (with support from John Elliot) and University of Goiás in Brazil (with support from Walkiria Toledo and Aline Magalhães Costa), on two occasions at the beginning and end of the trials: December 2016 (baseline; week 0) and March (post-intervention; week 12) for the England trial and June 2017 (baseline; week 0) and September 2017 (post-intervention; week 12) for the Brazil trial. Trial visits occurred in the morning between 7.00 and 11.30 am following overnight 8 hours fasting. Trial procedures at each visit are summarised in **Figure 2.2** below, followed by a description of all data collection methods. Participants were contacted weekly by telephone or email to maintain good communication and for adverse event reporting.
A lifestyle questionnaire was administered to assess for several cultural, behavioural and general lifestyle aspects. Participants were asked to complete the questionnaire by themselves during baseline visit, with a researcher from the D-SOL team (Marcela Mendes or Lara Nabuco) present to help with any eventual questions. Table 2.3 summarises the information collected from the lifestyle questionnaire. The full questionnaire can be found in Appendix I and included multi-choice as well as open questions regarding socio-demographics, behaviour towards sunlight exposure (sunscreen use and sunbathing habits), lifestyle and perceptions towards vitamin D and sunlight exposure.

The questionnaire was piloted with one adult Brazilian women living in England and one adult Brazilian women living in Brazil. The questionnaire was adapted to each country to make questions appropriate (i.e. Planning a trip between “December and March” for England

Figure 2.2 Study procedures completed during study visits. (pQCT: peripheral quantitative computed tomography; DXA: Dual-energy X-ray absorptiometry)

2.3.1 Lifestyle questionnaire

A lifestyle questionnaire was administered to assess for several cultural, behavioural and general lifestyle aspects. Participants were asked to complete the questionnaire by themselves during baseline visit, with a researcher from the D-SOL team (Marcela Mendes or Lara Nabuco) present to help with any eventual questions. Table 2.3 summarises the information collected from the lifestyle questionnaire. The full questionnaire can be found in Appendix I and included multi-choice as well as open questions regarding socio-demographics, behaviour towards sunlight exposure (sunscreen use and sunbathing habits), lifestyle and perceptions towards vitamin D and sunlight exposure.

The questionnaire was piloted with one adult Brazilian women living in England and one adult Brazilian women living in Brazil. The questionnaire was adapted to each country to make questions appropriate (i.e. Planning a trip between “December and March” for England or
“June and September” Brazil, respectively; “How many years living in England?” and “Where did you live before?” removed from Brazil trial questionnaire; pint as reference measure for England trial and replaced with 2 mugs for Brazil trial, both referring to 560ml).

Table 2.3 Information collected from the lifestyle questionnaire

<table>
<thead>
<tr>
<th>Health</th>
<th>Socio-demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous disease</td>
<td>Date of Birth</td>
</tr>
<tr>
<td>Previous medication/treatments</td>
<td>Nationality and country of birth</td>
</tr>
<tr>
<td>Currently or might be pregnant</td>
<td>Ethno-race R1</td>
</tr>
<tr>
<td>Ever fractured any bones</td>
<td>Skin type R2</td>
</tr>
<tr>
<td>Plans to get pregnant within next 12 months</td>
<td>Education level</td>
</tr>
<tr>
<td>Given birth in the last 3 months</td>
<td>Years living in England a</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>Previous country of residence a</td>
</tr>
<tr>
<td></td>
<td>Plans to travel during study period</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lifestyle</th>
<th>Behaviour towards sunlight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Activity Habit and Frequency</td>
<td>Body parts exposed</td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td>Sunscreen use</td>
</tr>
<tr>
<td>Habit of Smoking</td>
<td>Sunscreen SPF at home/during holiday</td>
</tr>
<tr>
<td>Milk consumption (per day)</td>
<td>Natural sunbathing habit</td>
</tr>
<tr>
<td>Egg, oily fish and fish consumption (per week)</td>
<td>Artificial sunbeds use</td>
</tr>
<tr>
<td>Supplement use (currently and within the last year)</td>
<td></td>
</tr>
</tbody>
</table>

* a Applicable to England trial participants only.

References: R1 Official ethno-racial categories for the Brazilian population by the Brazilian Institute of Geography and Statistics (111) R2 The Fitzpatrick Classification Scale for Skin Types (112).

2.3.2 Vitamin D and dietary intake

Dietary intake of participants, particularly vitamin D and calcium, was determined by 4 consecutive days of estimated diet diaries. Throughout the study, two diaries were collected for each participant (beginning – week 0, and end of the intervention – week 12). Participants were trained by the research team (Marcela Mendes or Lara Nabuco) on how to correctly complete the diary, using pre-filled forms and standard kitchen utensils for measurement standardization. They were also instructed to give as much detail as possible of every meal, including portion size. Records included days of the week and weekend as participants were instructed to always start on a Sunday, in order to be representative days.

Dietary intake data obtained from participants in the England trial were analyzed using the Nutritics® nutritional analysis software and those collected in Brazil were analyzed via the Dietwin® software. Nutritics® software is based on the UK Composition of Foods Integrated
Dataset (CoFID) including McCance and Widdowson 7th edition (70). The Dietwin® software database includes the Brazilian Food Composition Table (TACO) (113) and the food composition database from The Brazilian Institute of Geography and Statistics (IBGE), as well as the United States Department of Agriculture (USDA) food composition database.

Portions were estimated based on the description recorded and from consulting the relevant food portion sizes guides, namely Food Portion Sizes (Maff Handbook), 3rd Edition 2002 (114) and Table for Evaluation of Food Consumption in Domestic Measures (Tabela para Avaliação de Consumo Alimentar em Medidas Caseiras, in Portuguese), 5th Edition, 2004 (115). All diaries, from both countries, were analysed by Marcela Mendes. Gaps in quantity information were entered as the standard medium portion for the respective food.

### 2.3.3 Sunlight individual exposure

To determine individual exposure to ambient UVB radiation, participants were asked to wear individual UV exposure dosimeter polysulphone film badges (provided by Prof Anna Webb and Dr Richard Kift, University of Manchester) on their outer clothing. Participants were instructed to wear their dosimeters around the upper shoulder/chest region from sunrise to sunset for a full week (7 consecutive days) at both week 0 (baseline) and week 12 (post-intervention). They were instructed to store the dosimeter in the supplied envelope (of thick and dark material to prevent further UVB exposure during storage) and return on week 12 visit.

All dosimeters, for both the England and Brazil trials, were read at the University of Surrey by Marcela Mendes, prior to and after use, with a Cecil Aquarius CE7200 Double Beam Spectrophotometer (which has a CV <1%) at 330 nm, to detect change in absorbency. The amount of UV captured by each dosimeter badge was then translated to a standard erythematous dose (SED) using the following formula (15):

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

\[
\Delta A_{330} = \text{change in absorbance at 330 nm of the dosimeter from pre- to post-UVB exposure.}
\]

1 SED = 100 J m\(^{-2}\) of erythemal (sun burning) UV radiation and is generally used as a measure of UV exposure.
Participants’ individual sun exposure was also evaluated by a sunlight exposure diary equivalent to the 12 weeks (~90 days) of intervention. The records were performed daily through calendar code entry (Figure 2.3). Participants were trained on how to properly complete the records by the research team. Individuals were also instructed to record in the sun exposure diaries eventual trips out of the state or country of residency, specifying the exact location and dates of travel and return. All sun exposure diaries were then transcribed by Marcela Mendes to a standardized worksheet to obtain scores related to the most frequent individual exposure.

**Figure 2.3** Daily outdoor sun exposure diary: entry codes

<table>
<thead>
<tr>
<th>Code</th>
<th>Time spent outside today</th>
<th>Code</th>
<th>Sites exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Less than 15 minutes</td>
<td>A</td>
<td>Face only</td>
</tr>
<tr>
<td>2</td>
<td>Between 15 and 30 minutes</td>
<td>B</td>
<td>Hands and face</td>
</tr>
<tr>
<td>3</td>
<td>Between 30 minutes and 2 hours</td>
<td>C</td>
<td>Hands and face plus arms and/or legs</td>
</tr>
<tr>
<td>4</td>
<td>More than 2 hours</td>
<td>D</td>
<td>Hands and face plus arms and/or legs and some/all of the body</td>
</tr>
</tbody>
</table>

### 2.3.4 Ethno-race and skin type

Race and skin type were both self-reported via the lifestyle questionnaire (Appendix II). Race categories were based on the Brazilian ethno-race national demographic spectrum (Brazilian Institute of Geography and Statistics (111)), which includes: White, Black, Brown/Mixed (“Pardo” in Portuguese), Indigenous and “Yellow” (Asian-descendent). Participants were asked to indicate which category they most identified with.

Skin type categories were based on the Fitzpatrick validated classification for skin photo-types, which classifies the skin according to the ability of each skin type to tan under sun exposure and its sensitivity and tendency to turn red under the solar rays (Table 2.4) (112). Participants were asked to choose one category only that best represented the effect of sunlight exposure.
on their skins. For the purpose of this study, Fitzpatrick’s photo-types were combined into skin type categories as follow: Type I and II (white); Type III and IV (brown); Type V and VI (Black).

**Table 2.4** Fitzpatrick validated classification for skin photo-types

<table>
<thead>
<tr>
<th>Type</th>
<th>Skin natural colour</th>
<th>Skin reaction/sensitivity to sun exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>White</td>
<td>Always burns; Never tan; Very sensitive to the Sun</td>
</tr>
<tr>
<td>II</td>
<td>White</td>
<td>Always burning; Very little tan; Sun sensitive</td>
</tr>
<tr>
<td>III</td>
<td>Light brown</td>
<td>Burns moderately; Bronze moderately; Normal sensitivity to the Sun</td>
</tr>
<tr>
<td>IV</td>
<td>Moderate brown</td>
<td>Burns a little; Always tan; Normal sensitivity to the Sun</td>
</tr>
<tr>
<td>V</td>
<td>Dark brown</td>
<td>Rarely burns; Always tan; Not sensitive to the sun</td>
</tr>
<tr>
<td>VI</td>
<td>Black</td>
<td>Never burn; Totally pigmented; Insensitive to the Sun</td>
</tr>
</tbody>
</table>

### 2.3.5 Anthropometrics and blood pressure

Before data collection commenced, anthropometric methods were standardized between the different researchers involved in the study performing the measurements in each country (Saskia Wilson-Barnes, Dr Philippa Gibson and Hajrah Mukthar in England, and Sáskia Vaz, Amanda Oliveira and Marília Bohnen in Brazil, were trained by Marcela Mendes) in order to minimize inter-evaluator variations (116).

Participants were instructed to wear light clothing on the day of the trial visits, with gym clothes if possible, in order to minimize different added weight from clothing. For weight measurement, participants were asked to remove shoes, socks and heavy coats before stepping on the scale. For the England trial, weight to the nearest 0.1 kg and body fat was obtained using a Tanita Body Composition Analyser MC-180MA (Tanita Cooperatives, Tokyo, Japan). For the Brazil trial, weight was measured to the nearest 0.1 kg using a standard weighting scale (Balmak®) and body composition was determined via DXA scan.

Standing height was measured using a wall stadiometer, to the nearest 0.1 cm, with participants in an up-right posture and barefoot with heels close together and as close as possible to the wall (116).
Waist circumference was measured with a non-extendable standard measure tape, at the narrowest point of the torso, to the nearest 0.1 cm. If point could not be estimated, the level of the belly button was used as reference point.

For blood pressure measurements, participants were asked to sit and relax for 1 minute prior to taking the reading. They were positioned in an up right seated position with their arm supported on a levelled table. An automatic blood pressure monitor (Omron Healthcare Ltd, Kyoto, Japan) was used to obtain readings from the non-dominant arm. Blood pressure readings were repeated twice, with a one-minute interval between measures, and an average reading calculated.

2.3.6 Blood sample collection and processing

An overnight fasted (8 hours) blood sample was collected by venepuncture by trained phlebotomists at each visit (baseline and post-intervention). The following blood sample collection tubes were used for measurement of the specified metabolites:

- 10 ml BD Vacutainer® SST™ tube: serum 25(OH)D, calcium and albumin
- 6 ml BD Vacutainer® EDTA tube: plasma PTH

After collection, all blood samples were inverted 10 times as per manufacturer’s instructions before being processed by Marcela Mendes in the laboratory within the respective Clinical Investigation units of each University. For serum, the collected blood samples were left to clot for 1 hour at room temperature followed by centrifuge spinning at 3,000 g for 10 minutes at 4°C (England trial: Sigma 3-16K Centrifuge, SciQuip, Shropshire, UK; Brazil trial: Eppendorf™ 5702R Centrifuge, UK). For plasma, collected blood samples were spun immediately also at 3,000 g for 10 minutes at 4°C.

Processed serum and plasma samples were distributed into aliquots and stored at -80°C at the University of Surrey, prior to analysis. Samples collected in Brazil followed the exact same procedures and were temporarily stored at -80°C at the University of Goiás. The samples were then sent by air to the UK by World Courier Group Inc. UK Branch, arranged by Marcela
Mendes with the assistance of Dr Kathryn Hart, Dr Patrícia Botelho and MSc Anna Paula Gomes, to be stored at the University of Surrey as well, prior to analysis.

2.3.7 Laboratory analysis

All samples, from both countries, were analysed for 25(OH)D, PTH, serum calcium and serum albumin at Imperial College London, by Dr Emma Williams.

Serum 25(OH)D concentrations were determined by HPLC-MS/MS method on a Waters Acuity TQD using a PFP column following supported liquid extraction (SLE). Laboratory intra- and interassay CVs were 5.6% and 7.8%, respectively.

Due to the lack of global consensus as to the definition of vitamin D status, for the purpose of the D-SOL Study vitamin D status was defined as per Table 2.5 below. The references were chosen on the basis of current reference values followed in Brazil and in the United Kingdom.

<table>
<thead>
<tr>
<th>Serum 25(OH)D concentration</th>
<th>Status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 nmol/l</td>
<td>Deficiency</td>
<td>SACN (2016) (74)</td>
</tr>
<tr>
<td>25 – 49.9 nmol/l</td>
<td>Insufficiency</td>
<td>IOM (2011) (71)</td>
</tr>
<tr>
<td>50 – 74.9 nmol/l</td>
<td>Adequacy</td>
<td>IOM (2011)(71) /SBEM (2017) (91)</td>
</tr>
<tr>
<td>&gt; 75 nmol/l</td>
<td>Optimal</td>
<td>Endocrine Society (2011) (72)</td>
</tr>
</tbody>
</table>

SBEM: Brazilian Society of Endocrinology and Metabology

Calcium, albumin, and PTH concentrations were measured by using Abbott Architect methods. Serum calcium was measured by using an endpoint spectrophotometric reaction based on the o-cresolphthalein complexone methodology, and serum albumin was measured by using an endpoint spectrophotometric reaction based on the bromocresol green solution dye binding methodology. Serum calcium concentrations were adjusted for albumin concentrations. Plasma intact PTH was measured by in vitro chemiluminescent microparticle immunoassay (CMIA).
The manufacturer’s quoted inter-assay CV for calcium was <3%, for albumin <3.8% and for PTH 4%. Intact PTH reference values for adults range from 1 to 6.9 pmol/l (117). Serum calcium concentrations were adjusted for albumin concentrations and serum corrected calcium reference values for adults range from 2.1 to 2.6 mmol/L (117).

2.3.8 Bone parameters

For the England trial, a peripheral quantitative computed tomography (pQCT; XCT 2000, Stratec Medizintechnik GmbH, Pforzheim, Germany) was performed on the participant’s non-dominant forearm at the baseline visit only, to measure volumetric bone mineral density at the 4% and 66% radial site. pQCT was performed by the same experienced operator to scan all participants' tibias. The pQCT scan allows for separate measurements of vBMD and bone mineral content (BMC) for trabecular and cortical sites (4% and 66% sites, respectively, defined as the percentage of the tibia length from the distal to the proximal end). T-scores for total density and trabecular density were calculated using previously published reference data for white Caucasian European women (118) using the equation: $T$-score = \((\text{individual value} - \text{reference mean}) / \text{reference Standard Deviation (SD)}\).

For participants in Brazil, body composition (absolute and relative amount of lean and fat mass), whole body bone mineral density and lower spine and femur bone mineral density was measured via DXA (dual energy X-ray absorptiometry, GE Healthcare LunarTM DPX NT+152000, GE Medical Systems, Madison, Wisconsin, US) scan at baseline only. The same experienced operator performed all DXA scans with all participants. A DXA scan provides a two-dimensional estimate of an individual's bone mineral density (BMD) in the specific region of interest. T-scores of Brazilian participants for lumbar spine (L1-L4) and fêmur were automatically calculated by the DXA scan, which used as a reference white Caucasian Women (119,120).

The same cut-off values for T-score classification (i.e., T-score ≥−1.0 defined as low-risk score, T-score between −1.0 and −2.5 defined as moderate, and ≤−2.5 defined as high-risk score for osteoporosis) were applied for pQCT and DXA scans.
Exploring the concept of latitude-dependence of vitamin D status: impact of sunlight availability and individual UV radiation levels on serum 25-hydroxyvitamin D

3.1 Background

As previously discussed in Chapter 1, prolonged and severe vitamin D deficiency can lead to rickets in children and osteoporosis in adults. Vitamin D is naturally present in very few foods and in small quantities and the main source is considered to be casual exposure of the skin to the UVB portion of sunlight (290-350nm), which converts the molecule 7-dehydrocholesterol naturally present in the epidermis, to pre-vitamin D. Serum 25(OH)D concentrations reflect both photo-chemically synthesised vitamin D in the skin and dietary intake being, therefore, considered the best indicator of the long-term vitamin D status.

Populations living within the tropics, namely low latitude locations, are exposed to substantially higher levels of solar UV radiation throughout the year than those living in high latitude locations (17). The average ambient solar UV radiation in South America is about two to three times that in Northern Europe, rising even more with greater proximity to the equator. For instance, the maximum UV index (based on midday intensity in summer months) is 14 in Mid-west Brazil compared to only 7-8 in Southern England (121,122).

Recent reports of an increasing prevalence of low vitamin D concentrations in both low and high latitude locations show that vitamin D deficiency is rapidly becoming, if not already, a major global public health issue (50,99,104). Moreover, vitamin D deficiency and insufficiency are common in immigrant groups coming from low latitude countries to live in high latitude countries, being far more prevalent amongst ethnic groups compared to native populations in these locations (58).
More studies are needed to accurately estimate the prevalence of insufficient serum 25(OH)D concentrations in sunny countries. In order to develop effective vitamin D guidelines, we need to fully understand the actual impact of sunlight on 25(OH)D concentrations, based not only on UV radiation availability (latitude) but also individual UV radiation exposure, as well as the relative contribution of key influential factors, such dietary intake, adiposity, skin pigmentation and lifestyle.

### 3.2 Objectives

- **Objective 1:**
  To compare the intensity of individual UV radiation levels between adult women living in England and adult women living in Brazil, and investigate the association with circulating 25(OH)D concentrations.

- **Objective 2:**
  To evaluate the habitual dietary Vitamin D and calcium intakes and associations with circulating 25(OH)D concentrations in adult women living in England and adult women living in Brazil.

- **Objective 3:**
  To determine the prevalence of vitamin D deficient, insufficient, adequate and optimal status in adult women living in England and adult women living in Brazil.

- **Objective 4:**
  To determine the relationship between serum 25(OH)D and plasma PTH concentrations and to investigate whether there is a threshold for serum 25(OH)D concentrations where a plateau in PTH is evident and useful in setting a cut-off threshold for vitamin D adequacy for this population.

- **Objective 5:**
  To explore the relationship between serum 25(OH)D concentration, vitamin D intake, individual sunlight exposure and influential factors.
3.3 Methods

The study recruited participants at the University of Surrey (51.°N) England, from October to December 2016 and participants at the University of Goiás (16°S), Brazil, from April to June 2017. All participants at commencement of the study provided a written informed consent. Full clinical and methodological study details, including participant recruitment, randomization and data collection, are described in Chapter 2.

3.3.1 Statistical analysis

Statistical analysis of the data was undertaken using SPSS software for Windows (version 25.0; IBM Corp, Armonk, NY).

Data was tested for normal distribution using the Kolmogorov-Smirnov tests. Non-normally distributed variables were log transformed and reported in the original scale. Non-parametric tests were used when log transforming did not normalise the data. Descriptive statistics were determined for all variables. Continuous variables are presented as mean ±SD for normally distributed variables or as median (25%, 75% percentiles) for not normally distributed. For categorical variables, frequency and percentage were reported.

Baseline characteristics (age, weight, BMI, waist circumference, dietary intakes, UVB exposure and biomarkers) were compared between countries, by independent t-tests, or Mann-Whitney U tests for non-normally distributed data. The distribution of skin type and BMI classification were compared between countries using chi squared tests.

For the whole sample and in each country separately, Pearson correlation, or the corresponding non-parametric Spearmans rho correlation tests were used to investigate the relationship between circulating serum 25(OH)D and individual UV radiation levels, dietary intakes of vitamin D and calcium, and anthropometric measures. Mean circulating serum 25(OH)D concentration was compared between different aspects of lifestyle, individual characteristics and individual UV radiation levels using independent t-tests, or Mann-Whitney U tests for non-normally distributed data; or one-way ANOVA with post-hoc Tukey tests, or Kruskall-Wallis
for non-normally distributed data. Standard linear regression models were run to investigate the predictive ability of individual daily sunlight exposure on circulating serum 25(OH)D concentrations.

A p value of <0.05 was considered significant. For comparisons between groups, effect size based on eta squared was defined as: 0.01 as a small effect, 0.06 as a medium effect and 0.14 as a large effect (123).
3.4 Results

3.4.1 Study participants

A total of 335 adult Brazilian women were screened for the study, 148 in England and 187 in Brazil, of which: 131 were excluded based on the exclusion criteria; 53 decided not to participate after the screening process and 15 did not attend their baseline visit. Reasons for exclusion at screening are detailed in Figure 3. In the Brazil cohort one participant did not have valid laboratory results, and was therefore excluded from database.

Of the participants enrolled for the D-SOL study after the screening process, 135 participants were included in this cross-sectional analysis (n = 56 in England and 79 in Brazil). In the England cohort five participants were post-menopausal. There were no differences between analyses including or excluding the 5 post-menopausal women, and therefore, only analyses including these participants are reported here.

Figure 3.1 Flow diagram of participant enrolment
3.4.2 Baseline characteristics

3.4.2.1 Socio-demographic and anthropometric characteristics

In the overall sample the median age was 29 (25, 35) years. Amongst the 135 participants, 63% identified themselves as white and 33.3% as Brown (mixed). The proportion of white women in the England cohort was significantly higher ($p=0.012$) while in the Brazil cohort there was an even distribution between white and brown (51.9% and 44.3%, respectively). In respect to skin type classification, the majority of participants classified themselves as type III and IV (63% overall, 66.1% of England and 60.8% of Brazil residents), which corresponds to light and moderate brown in the Fitzpatrick scale, with no differences in proportions between the two countries (Table 3.1). The majority of women had a university degree in both countries (70.4% overall), with a higher proportion in the England cohort ($p=0.014$). Two thirds of the England dwelling participants had been living in Southern England for more than 2 years (72.7%) (Table 3.1).

Table 3.1 Socio-demographic characteristics of adult Brazilian women overall and by country of residence (n=135)

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>England (n = 56)</th>
<th>Brazil (n = 79)</th>
<th>$p^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29 (25, 35)</td>
<td>33 (29, 41)</td>
<td>27 (24, 31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ethno-race [n(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>85 (63%)</td>
<td>44 (78.6%)</td>
<td>41 (51.9%)</td>
<td>0.012a</td>
</tr>
<tr>
<td>Black</td>
<td>3 (2.2%)</td>
<td>1 (1.8%)</td>
<td>2 (2.5%)</td>
<td></td>
</tr>
<tr>
<td>Brown (mixed)</td>
<td>45 (33.3%)</td>
<td>10 (17.9%)</td>
<td>35 (44.3%)</td>
<td></td>
</tr>
<tr>
<td>Yellow (Japanese-descendant)</td>
<td>1 (0.7%)</td>
<td>0</td>
<td>1 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Indigenous (native Indian)</td>
<td>1 (0.7%)</td>
<td>1 (1.8%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Skin type [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>0.589a</td>
</tr>
<tr>
<td>Type I and II</td>
<td>42 (31.1%)</td>
<td>17 (30.4%)</td>
<td>25 (31.6%)</td>
<td></td>
</tr>
<tr>
<td>Type III and IV</td>
<td>85 (63%)</td>
<td>37 (66.1%)</td>
<td>48 (60.8%)</td>
<td></td>
</tr>
<tr>
<td>Type V and VI</td>
<td>8 (5.9%)</td>
<td>2 (3.6)</td>
<td>6 (7.6)</td>
<td></td>
</tr>
<tr>
<td>Education level [n(%)]</td>
<td></td>
<td></td>
<td></td>
<td>0.014a</td>
</tr>
<tr>
<td>Secondary school (&gt; 16 yrs of age)</td>
<td>3 (2.2%)</td>
<td>1 (1.8%)</td>
<td>2 (2.5%)</td>
<td></td>
</tr>
<tr>
<td>A levels (18 yrs of age)</td>
<td>37 (27.4%)</td>
<td>8 (14.3%)</td>
<td>29 (36.7%)</td>
<td></td>
</tr>
<tr>
<td>University Degree</td>
<td>95 (70.4%)</td>
<td>47 (83.9%)</td>
<td>48 (60.8%)</td>
<td></td>
</tr>
<tr>
<td>Years living in England [n(%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>N/A</td>
<td>5 (9.1%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1 – 2 years</td>
<td>10 (18.2%)</td>
<td>10 (18.2%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>&gt; 2 years</td>
<td>50 (72.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values are median (25th, 75th percentile) or n (%). 2 Statistical analysis: Mann-Whitney U unless otherwise stated; a Pearson Chi Squares. References: R1 Official ethno-racial categories for the Brazilian population by the Brazilian Institute of Geography and Statistics (111) R2 The Fitzpatrick Classification Scale for Skin Types (112). N/A: Not Applicable
In the overall sample (n=135), median BMI was 23.9 (20.9, 26.73) kg/m² and a total of 61.5% of the women had a BMI within the healthy range of <25 kg/m², while 23.7% were overweight (25 – 29.99 kg/m²) and 14.8% were obese (>30 kg/m²) (Table 3.1).

Brazilian women living in England were older, heavier and had a greater waist circumference than those living in Brazil (p<0.01). There were no significant differences between Brazilian women living in England and in Brazil for BMI classification distributions although, in line with the weight data, the mean BMI was significantly greater for those residing in England (Table 3.2).

Table 3.2 Baseline anthropometric characteristics of adult Brazilian women overall and by country of residence (n=135)¹

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>England (n = 56)</th>
<th>Brazil (n = 79)</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>62.95 (55.85, 71.80)</td>
<td>67.25 (60.77, 72.95)</td>
<td>60.15 (54.1, 71.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>75.70 (68.20, 87.70)</td>
<td>86.05 (76.40, 93.77)</td>
<td>70.4 (66.1, 77.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>23.9 (20.97, 26.73)</td>
<td>24.79 (22.69, 28.09)</td>
<td>23.34 (20.37, 26.38)</td>
<td>0.009</td>
</tr>
<tr>
<td>BMI category [n (%)]</td>
<td>83 (61.5%)</td>
<td>29 (51.8%)</td>
<td>54 (68.3%)</td>
<td>0.140a</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>32 (23.7%)</td>
<td>16 (28.6%)</td>
<td>16 (20.3%)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>20 (14.8%)</td>
<td>11 (19.6%)</td>
<td>9 (11.4%)</td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>35.46 ± 8.41</td>
<td>30.96 ± 5.84</td>
<td>38.65 ± 8.52</td>
<td>&lt;0.001b</td>
</tr>
</tbody>
</table>

¹ Values are median (25th, 75th percentile), mean ± SD or n (%).
² Statistical analysis: Mann-Whitney U unless otherwise stated; a Chi Squares; b Independent t-test
³ Measurements derived from different methodologies (England: bio-impedance; Brazil: DXA scan)
References: R¹ World Health Organization BMI classification (124)

3.4.2.2 Lifestyle characteristics

Overall, around half of the women reported being physically active (59.3%), of which most reported exercising 3 times a week or more (75%). Very few participants smoked (6.7%) and over half reported habitually consuming alcohol (57%). There were no significant differences in any of the characteristics or reported behaviours between women living in England and women living in Brazil.
Overall, nearly a third of the participants reported never consuming milk (28.7%) and nearly a quarter reported never consuming oily fish (22.9%). There were differences in the frequency of consumption of milk and oily fish between the two countries. Around 30% of women living in England reported drinking more than 2 mugs of milk per day compared to 10% in Brazil ($p=0.001$), and over half (51.8%) in England consumed oily fish at least once a week compared to only 21.6% in Brazil ($p=0.005$). Overall, around half of the women across both countries (45.2%) reported consuming eggs at least 2-5 times a week and 77.1% reported never consuming liver (Table 3.3).

In the total sample, the majority of participants (70%) had not taken supplements containing vitamin D or fish/fish liver oil supplements within the last 12 months, although the proportion was significantly higher amongst women living in Brazil (68.4% versus 57.2% for those living in England, $p=0.002$) (Table 3.3).
Table 3.3 Lifestyle characteristics of adult Brazilian women overall and by country of residence (n=135)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>England (n = 56)</th>
<th>Brazil (n = 79)</th>
<th>( p^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Activity(^8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>55 (40.7%)</td>
<td>32 (57.1%)</td>
<td>31 (39.2%)</td>
<td>0.673</td>
</tr>
<tr>
<td>Yes</td>
<td>80 (59.3%)</td>
<td>24 (42.9%)</td>
<td>48 (60.8%)</td>
<td></td>
</tr>
<tr>
<td>Physical Activity Frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 x</td>
<td>18 (22.5%)</td>
<td>7 (21.9%)</td>
<td>11 (26.2%)</td>
<td>0.506</td>
</tr>
<tr>
<td>3-4 x</td>
<td>42 (52.5%)</td>
<td>19 (59.3%)</td>
<td>23 (54.7%)</td>
<td></td>
</tr>
<tr>
<td>5-7 x</td>
<td>14 (17.5%)</td>
<td>7 (18.8%)</td>
<td>8 (19%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>6 (7.5%)</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td></td>
<td></td>
<td></td>
<td>0.467</td>
</tr>
<tr>
<td>No</td>
<td>58 (43%)</td>
<td>34 (60.7%)</td>
<td>36 (45.6%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>77 (57%)</td>
<td>22 (39.3%)</td>
<td>43 (54.4%)</td>
<td></td>
</tr>
<tr>
<td>Smoke</td>
<td></td>
<td></td>
<td></td>
<td>0.375</td>
</tr>
<tr>
<td>No</td>
<td>126</td>
<td>5 (8.9%)</td>
<td>75 (94.9%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>(93.3%)</td>
<td>51 (91.1%)</td>
<td>4 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>Milk consumption (per day)</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Never</td>
<td>39 (28.7%)</td>
<td>13 (23.2%)</td>
<td>28 (35.5%)</td>
<td></td>
</tr>
<tr>
<td>Less than 1 mug (&lt; 280 ml)</td>
<td>31 (23%)</td>
<td>17 (30.4%)</td>
<td>14 (17.7%)</td>
<td></td>
</tr>
<tr>
<td>1 mug (280 ml)</td>
<td>38 (28.1)</td>
<td>9 (16.1%)</td>
<td>29 (36.7%)</td>
<td></td>
</tr>
<tr>
<td>2 mugs (560 ml)</td>
<td>21 (15.6%)</td>
<td>13 (21.3%)</td>
<td>8 (10.1%)</td>
<td></td>
</tr>
<tr>
<td>More than 2 mugs (&gt; 560 ml)</td>
<td>4 (3%)</td>
<td>4 (7.1%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Egg consumption (per week)</td>
<td></td>
<td></td>
<td></td>
<td>0.203</td>
</tr>
<tr>
<td>Never</td>
<td>4 (2.9%)</td>
<td>1 (1.8%)</td>
<td>3 (3.8%)</td>
<td></td>
</tr>
<tr>
<td>Less than once</td>
<td>21 (15.6%)</td>
<td>9 (16.1%)</td>
<td>12 (15.2%)</td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>29 (21.5%)</td>
<td>17 (30.4%)</td>
<td>12 (15.2%)</td>
<td></td>
</tr>
<tr>
<td>2-5 times</td>
<td>61 (45.2%)</td>
<td>24 (42.9%)</td>
<td>37 (46.8%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 times</td>
<td>20 (14.8%)</td>
<td>5 (8.9%)</td>
<td>15 (19%)</td>
<td></td>
</tr>
<tr>
<td>Oily fish consumption (per week)</td>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>Never</td>
<td>31 (22.9%)</td>
<td>10 (17.9%)</td>
<td>20 (25.3%)</td>
<td></td>
</tr>
<tr>
<td>Less than once</td>
<td>58 (43%)</td>
<td>17 (30.4%)</td>
<td>41 (51.9%)</td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>33 (24.4%)</td>
<td>21 (37.5%)</td>
<td>12 (15.2%)</td>
<td></td>
</tr>
<tr>
<td>2-5 times</td>
<td>12 (8.9%)</td>
<td>8 (14.3%)</td>
<td>4 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 times</td>
<td>1 (0.7%)</td>
<td>0</td>
<td>1 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Liver consumption (per week)</td>
<td></td>
<td></td>
<td></td>
<td>0.520</td>
</tr>
<tr>
<td>Never</td>
<td>(77.1%)</td>
<td>10 (17.9%)</td>
<td>53 (73.4%)</td>
<td></td>
</tr>
<tr>
<td>Less than once</td>
<td>28 (20.7%)</td>
<td>17 (30.4%)</td>
<td>19 (24.1%)</td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>2 (1.5%)</td>
<td>21 (37.5%)</td>
<td>1 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>2-5 times</td>
<td>1 (0.7%)</td>
<td>8 (14.3%)</td>
<td>1 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5 times</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Supplement use (within the last year)</td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>None</td>
<td>86 (70%)</td>
<td>32 (57.2%)</td>
<td>54 (68.4%)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>15 (11.1%)</td>
<td>3 (5.4%)</td>
<td>12 (15.2%)</td>
<td></td>
</tr>
<tr>
<td>Fish/fish liver oil</td>
<td>3 (2.2%)</td>
<td>2 (3.6%)</td>
<td>1 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Fish/fish liver oil with vitamin D</td>
<td>14 (10.4%)</td>
<td>5 (8.9%)</td>
<td>9 (11.4%)</td>
<td></td>
</tr>
<tr>
<td>Multivitamins with vitamin D</td>
<td>13 (9.6%)</td>
<td>12 (21.4%)</td>
<td>1 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Calcium with vitamin D</td>
<td>4 (3%)</td>
<td>2 (3.6%)</td>
<td>2 (2.5%)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Values are median (25\(^{th}\), 75\(^{th}\) percentile) or n (%). \(^2\)Statistical analysis: Chi Squares. \(^8\) Amongst participants who said “Yes” to previous item.
3.4.2.3 Sun exposure behaviour

The patterns of all sunlight-related behaviours reported by the life-style questionnaire were significantly different between women living in England and women living in Brazil (all \( p \leq 0.05 \)), except for sunscreen use in general and sun protection factor (SPF) during holidays (both \( p > 0.05 \)) (Table 3.4).

In regards to body part exposed when out in the sun reported in the life-style questionnaire, more than half (58.9%) of women in England reported usual exposure of hands and face only, while in Brazil most women (70.9%) reported the habit of exposing hands and face plus arms and/or legs (\( p < 0.001 \)).

In total, around 70% if participants reported habitual use of sunscreen, although for use at home, within those living in England SPF of 15 or 20 was more common (52.3%) while in Brazil nearly all participants (95.4%) reported using SPF of 30, 40 or over (\( p = 0.003 \)). The proportion of participants that reported the habit of natural sunbathing was higher amongst women living in England than those living in Brazil (41.1% and 25.3% respectively, \( p = 0.05 \)), and only 3 women in England reported having ever used an artificial sunbed compared to none in Brazil.
Table 3.4 Behaviour towards sun exposure of adult Brazilian women overall and by country of residence (n=135)\(^1\)

<table>
<thead>
<tr>
<th>Body parts exposed</th>
<th>All</th>
<th>England (n = 56)</th>
<th>Brazil (n = 79)</th>
<th>p(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face only</td>
<td>1 (0.7%)</td>
<td>1 (1.8%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hands and face</td>
<td>48 (35.6%)</td>
<td>33 (58.9%)</td>
<td>15 (19%)</td>
<td></td>
</tr>
<tr>
<td>Hands/face + arms and/or legs</td>
<td>71 (52.6%)</td>
<td>15 (26.8%)</td>
<td>56 (70.9%)</td>
<td></td>
</tr>
<tr>
<td>Hands/face + arms/legs + torso</td>
<td>15 (11.1%)</td>
<td>7 (12.5%)</td>
<td>8 (10.1%)</td>
<td></td>
</tr>
<tr>
<td>sunscreen use</td>
<td>0.253</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>41 (30.4%)</td>
<td>14 (25%)</td>
<td>27 (34.2%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>94 (69.6%)</td>
<td>42 (75%)</td>
<td>52 (65.8%)</td>
<td></td>
</tr>
<tr>
<td>SPF at home(^\d)</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>11 (11.7%)</td>
<td>10 (23.8%)</td>
<td>1 (2.27%)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>14 (14.8%)</td>
<td>12 (28.5%)</td>
<td>1 (2.27%)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>30 (31.9%)</td>
<td>6 (14.2%)</td>
<td>23 (54.5%)</td>
<td></td>
</tr>
<tr>
<td>40 or over</td>
<td>32 (34.0%)</td>
<td>14 (32.5%)</td>
<td>18 (40.9%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>7 (7.4%)</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>SPF at holidays(^\dd)</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>8 (8.4%)</td>
<td>7 (16.7%)</td>
<td>1 (0.19%)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>3 (3.2)</td>
<td>2 (4.7%)</td>
<td>1 (0.19%)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>34 (35.8%)</td>
<td>17 (40.6%)</td>
<td>17 (32.6%)</td>
<td></td>
</tr>
<tr>
<td>40 or over</td>
<td>39 (41%)</td>
<td>15 (35.8%)</td>
<td>24 (46.1%)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>11 (11.6%)</td>
<td>1 (2.1%)</td>
<td>9 (21.4%)</td>
<td></td>
</tr>
<tr>
<td>natural sunbathing habit</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>92 (68.1%)</td>
<td>33 (58.9%)</td>
<td>59 (74.7%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43 (31.9%)</td>
<td>23 (41.1%)</td>
<td>20 (25.3%)</td>
<td></td>
</tr>
<tr>
<td>artificial sunbeds use</td>
<td>0.036</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>131 (97%)</td>
<td>52 (92.9%)</td>
<td>79 (100%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (2.2%)</td>
<td>3 (5.4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>1 (0.8%)</td>
<td>1 (1.7%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Values are median (25\(^{th}\), 75\(^{th}\) percentile) or n (%).\(^2\) Statistical analysis: Pearson Chi Squares.\(^\d\) Amongst participants who said “Yes” to previous item. SPF: Sun Protection Factor

From the 90-days sun diary data, during winter months in England 22.9% of participants spent no more than 15 minutes outdoor per day on most days compared to 30.5% in Brazil. The majority in both countries spent 15 – 30 minutes outside on most days (56.3% in England and 49.2% in Brazil). There were no statistically significant difference in proportions between the two countries (p=0.712) in total time spent outdoor per day on most days. There were also no significant differences in body part exposed on most days between the two countries. 38.8 % of women living in England and 50.8% of women living in Brazil reported exposing Hands
and face, and 49% in England and 40.7% in Brazil reported exposing hands, face, arms and/or legs, on most days. (Table 3.5)

### Table 3.5 Habitual exposure to sunlight by country

<table>
<thead>
<tr>
<th>Total time outdoor / day</th>
<th>England (n=48)</th>
<th>Brazil (n=59)</th>
<th>p value&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15 minutes</td>
<td>11 (22.9%)</td>
<td>18 (30.5%)</td>
<td>0.712</td>
</tr>
<tr>
<td>15 – 30 minutes</td>
<td>27 (56.3%)</td>
<td>29 (49.2%)</td>
<td></td>
</tr>
<tr>
<td>30 minutes – 2 hours</td>
<td>7 (12.5%)</td>
<td>10 (16.9%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 2 hours</td>
<td>3 (5.4%)</td>
<td>2 (3.4%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Body part exposed when outdoor</th>
<th>England (n=48)</th>
<th>Brazil (n=59)</th>
<th>p value&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face only</td>
<td>6 (12.2%)</td>
<td>4 (6.8%)</td>
<td>0.396</td>
</tr>
<tr>
<td>Hands and face</td>
<td>19 (38.8%)</td>
<td>30 (50.8%)</td>
<td></td>
</tr>
<tr>
<td>Hands and face + arms and/or legs</td>
<td>24 (49.0%)</td>
<td>24 (40.7%)</td>
<td></td>
</tr>
<tr>
<td>Hands and face + arms and/or legs + torso</td>
<td>0</td>
<td>1 (1.7%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are median (25<sup>th</sup>, 75<sup>th</sup> percentile) or n (%).<sup>2</sup> Statistical analysis: Chi squared

### 3.4.3 Dietary vitamin D and calcium intakes

Overall (n=119), mean habitual vitamin D dietary intake was 2.45 ± 1.91 μg/day and mean calcium intake was 625.05 ± 310.73 mg/day. Vitamin D and calcium intakes were significantly higher in English residents compared to Brazilian residents (p<0.001 and p=0.003, respectively).

In total (n=119), 99.2% had intakes below the EAR of 10 μg/day proposed by SACN (74) and by the IOM (71), with only one participant recording intakes above this threshold. Overall, 21.8% had dietary vitamin D intakes below 1 μg/day and 100% had intakes below the RDA of 15 μg/day. Mean dietary vitamin D intakes were well below the recommended EAR value as shown in Figure 3.2.
For calcium, in the overall sample 72.8\% had dietary intakes below the RNI of 700 mg/day (74), while only 8.8\% met the 800 mg/day EAR reference (71). Mean dietary intake of women living in Brazil was below both recommendation references, while mean intake for those living in England was above the RNI value (Figure 3.3).

**Figure 3.2** Mean daily vitamin D dietary intake in each country, compared to recommended values.

**Figure 3.3** Mean daily calcium dietary intake in each country, compared to recommended values.
3.4.4 Individual UV radiation levels

The average daily individual UV radiation levels at the beginning of winter measured by polyshulphone dosimeter badges are shown in Figure 3.4 for all participants, labelled by country of residence. Values are expressed in units of standard erythema dose (SED) (28). Individual UV radiation levels differed significantly between the two countries with concentrations ranging from 0.0031 to 0.0984 SED, for English residents and from 0.3283 to 12.0393 SED for Brazilian residents. Mean daily individual UV radiation levels for England residents were significantly lower than for Brazil residents (0.035 ± 0.026 and 1.75 ± 2.32 SED, respectively, p<0.001). All England dwelling participants recorded daily exposure levels of less than 1 SED. For those living in Brazil, around half of participants (53.6%) recorded levels of less than 1 SED.

![Graph showing individual UV radiation levels for women living in England and Brazil](image)

Blue and green hashed lines represent mean daily individual UV radiation level for women living in England (measured between October to March) and Brazil (measured between June to September), respectively.

**Figure 3.4** Average daily individual UV radiation levels for women living in England (n=46) and women living in Brazil (n=69).
3.4.5 Vitamin D Status

3.4.5.1 Serum 25(OH)D concentrations

Mean serum 25(OH)D concentration of England residents was significantly lower than Brazil residents (36.1 ± 14.7 nmol/l and 75 ± 22.1 nmol/l, respectively p<0.001). The statistical significance remained after controlling for daily UV radiation level, age, BMI and waist circumference (ANCOVA, p<0.001).

Figure 3.5 shows the difference in serum 25OHD concentrations between the two countries, with concentrations ranging from 5.0 to 73.5 nmol/l within participants living in England and from 36.2 to 148.6 nmol/l within those living in Brazil.

![Graph showing serum 25(OH)D concentrations for women living in England (n=56) and women living in Brazil (n=79).]

Orange, red and black hashed lines represent thresholds of 25 (deficiency), 50 (insufficiency) and 75 (optimal) nmol/l, respectively.

Figure 3.5 Serum 25(OH)D concentrations for women living in England (n=56) and women living in Brazil (n=79).
3.4.5.2 Proportion of women with Deficient, Insufficient, Adequate and Optimal Baseline Vitamin D Status

The proportions of women with deficient and insufficient levels were significantly higher in those living in England than in Brazil (p<0.001). Amongst women living in England 25% were vitamin D deficient, with serum 25(OH)D concentrations below 25 nmol/l, while there were no participants with concentrations below this threshold amongst those living in Brazil (Figure 3.6). There were no participants living in England with concentrations above 75 nmol/l, while half (50.6%) of the participants in Brazil presented optimal levels, above this threshold. The majority (82.1%) of women living in England and 11.4% of those living in Brazil had concentrations below the insufficiency cut off value of 50 nmol/l.

Figure 3.6 Proportion of women (total, England and Brazil trials) with deficient (<25 nmol/l), insufficient (25 – 49.9 nmol/l), adequate (50 – 74.9 nmol/l) and optimal (>75 nmol/l) serum 25OHD concentrations at baseline.
3.4.6 Factors affecting Serum 25(OH)D Concentrations

3.4.6.1 Socio-demographic Characteristics

There were no significant differences in mean serum 25(OH)D concentrations between ethno-race, skin type nor education level (all p> 0.081). Amongst England residents, those living in Southern England for more than 2 years had significantly lower 25(OH)D concentrations than those that had recently moved to the UK (less than a year) (p=0.039) (Table 3.6).

Table 3.6 Associations between socio-demographic characteristics and serum 25(OH)D concentrations in nmol/l

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>England</th>
<th>Brazil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethno-race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>85</td>
<td>55.6± 25.8</td>
<td>44</td>
</tr>
<tr>
<td>Black</td>
<td>3</td>
<td>43.1±15.3</td>
<td>1</td>
</tr>
<tr>
<td>Brown (mixed)</td>
<td>45</td>
<td>66.3±29.5</td>
<td>10</td>
</tr>
<tr>
<td>Yellow (Japanese-descendant)</td>
<td>1</td>
<td>59.5</td>
<td>0</td>
</tr>
<tr>
<td>Native Indian</td>
<td>1</td>
<td>42.9</td>
<td>1</td>
</tr>
<tr>
<td>Skin type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>42</td>
<td>61.0±24.9</td>
<td>17</td>
</tr>
<tr>
<td>III and IV</td>
<td>85</td>
<td>57.4±28.9</td>
<td>37</td>
</tr>
<tr>
<td>V and VI</td>
<td>8</td>
<td>63.0±21.0</td>
<td>2</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary school</td>
<td>3</td>
<td>71.3±29.4</td>
<td>1</td>
</tr>
<tr>
<td>A levels</td>
<td>37</td>
<td>60.3±29.2</td>
<td>8</td>
</tr>
<tr>
<td>University Degree</td>
<td>95</td>
<td>57.9±28.7</td>
<td>47</td>
</tr>
<tr>
<td>Years living in</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>England</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>5</td>
<td>51.0±14.9a</td>
<td></td>
</tr>
<tr>
<td>1 – 2 years</td>
<td>10</td>
<td>37.4±11.36</td>
<td></td>
</tr>
<tr>
<td>&gt; 2 years</td>
<td>40</td>
<td>33.5±14.6a</td>
<td></td>
</tr>
</tbody>
</table>

1Values: mean ± SD
2Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same column with same superscript letters are significantly different (* p = 0.032).
3.4.6.2 Age and adiposity

Overall (n=135), serum 25(OH)D concentration was significantly negatively correlated with age ($r = -0.282$, $p = 0.001$, Figure 3.7), weight ($r = -0.185$, $p = 0.031$, Figure 3.8), waist circumference ($r = -0.361$, $p < 0.001$, Figure 3.9), and a trend for a negative association with BMI ($r = -0.169$, $p = 0.052$), but not body fat ($r = -0.052$, $p = 0.706$). However, statistical significance was lost for all correlations when controlling for individual UV radiation level (all $p > 0.270$).

Figure 3.7 Association between age and serum 25(OH)D concentrations in overall sample (n=135)
Figure 3.8 Association between weight and serum 25(OH)D concentrations in overall sample (n=135)

Country of Residence
- ENGLAND
- BRAZIL

$r = -0.185$
$p = 0.031$

Figure 3.9 Association between waist circumference and serum 25(OH)D concentrations in overall sample (n=135)

Country of Residence
- ENGLAND
- BRAZIL

$r = -0.361$
$p < 0.001$
Within each country, no correlations were found between serum 25(OH)D concentration and age nor adiposity (weight, BMI, body fat or waist circumference) (all \( p > 0.406 \)) either before and after controlling for individual UV radiation level (Table 3.7). There were also no significant differences in mean age, weight, waist circumference and BMI between vitamin D status groups, in either country (all \( p > 0.06 \)).

**Table 3.7** Correlations between age, weight, BMI and waist circumference with serum 25(OH)D concentrations, after controlling for individual UV radiation level (n=135)

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>England</th>
<th>Brazil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( p )</td>
<td>( r )</td>
</tr>
<tr>
<td>Age</td>
<td>0.002</td>
<td>0.938</td>
<td>0.061</td>
</tr>
<tr>
<td>Weight</td>
<td>-0.044</td>
<td>0.643</td>
<td>-0.039</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.090</td>
<td>0.342</td>
<td>-0.082</td>
</tr>
<tr>
<td>Waist circ.</td>
<td>-0.104</td>
<td>0.269</td>
<td>-0.013</td>
</tr>
<tr>
<td>Body fat ( ^\dagger )</td>
<td>0.216</td>
<td>0.210</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^\dagger\)Partial correlations; \(^\dagger\) Measurements derived from different methodologies (England: bio-impedance; Brazil: DXA scan).

Overall, women younger than 30 years of age had significantly higher mean 25(OH)D concentrations than those aged 30 – 44 years (64.7 ± 27.4 and 51.6 ± 27.2 nmol/l, \( p=0.027 \)), however no significant differences amongst age groups were observed within each country (Table 3.8). There were no differences in mean 25(OH)D concentrations amongst healthy, overweight and obese women, either overall or within each country (all \( p > 0.08 \)).
### Table 3.8 Age and adiposity association with serum 25(OH)D concentrations

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>All 25(OH)D</th>
<th>England 25(OH)D</th>
<th>Brazil 25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3.8</td>
<td>0.030</td>
<td>0.761</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>64.7 ± 27.4</td>
<td>13.2 ± 3.1</td>
<td>74.1 ± 24.4</td>
</tr>
<tr>
<td>30 – 44 &gt;</td>
<td>51.6 ± 27.2</td>
<td>15.7 ± 2.9</td>
<td>78.2 ± 18.0</td>
</tr>
<tr>
<td>44</td>
<td>54.8 ± 21.1</td>
<td>14.9 ± 4.9</td>
<td>72.5 ± 12.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI</th>
<th>All 25(OH)D</th>
<th>England 25(OH)D</th>
<th>Brazil 25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3.4</td>
<td>0.088</td>
<td>0.163</td>
</tr>
<tr>
<td>Healthy</td>
<td>62.9 ± 28.7</td>
<td>36.3 ± 12.8</td>
<td>77.2 ± 24.4</td>
</tr>
<tr>
<td>Overweight</td>
<td>52.3 ± 19.7</td>
<td>39.0 ± 14.7</td>
<td>65.6 ± 14.6</td>
</tr>
<tr>
<td>Obese</td>
<td>52.4 ± 28.9</td>
<td>19.3 ± 5.8</td>
<td>78.0 ± 13.5</td>
</tr>
</tbody>
</table>

1Values: mean ± SD

2Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same column with same superscript letters are significantly different (\(p < 0.027\)).

#### 3.4.6.3 Lifestyle

There were no significant differences in serum 25(OH)D concentrations between individuals who were physically active and those who were not, nor between those who consumed alcohol and those who did not (all \(p > 0.231\)) (Table 3.10).

Overall, those consuming eggs for more than five times per week had significantly higher 25(OH)D concentrations (77.5±30.5 nmol/l) compared to those consuming less than once or once a week (51.4±23.3 nmol/l, \(p=0.014\) and 47.1±23.1 nmol/l, \(p=0.001\), respectively). There was also a difference in mean concentrations amongst different frequencies of consumption of milk, although post-hoc tests did not identify which groups differed significantly (\(p=0.009\)). The same was observed for oily fish consumption for the overall sample, and a more clear difference was observed within England participants, with those consuming 2-5 times per week having significantly higher concentrations (48.4±21.2 nmol/l) compared to those consuming less than once a week (31.6±16.2 nmol/l, \(p=0.034\)). There were no significant differences between different frequencies of liver consumption (all \(p > 0.464\)) (Table 3.9). There were no significant differences in 25(OH)D concentrations amongst different supplement intakes (all \(p > 168\)) (Table 3.9).
### Table 3.9 The association between lifestyle characteristics and with serum 25(OH)D concentrations

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th></th>
<th>England</th>
<th></th>
<th>Brazil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n 25(OH)D</td>
<td>p²</td>
<td>n 25(OH)D</td>
<td>p²</td>
<td>n 25(OH)D</td>
<td>p²</td>
</tr>
<tr>
<td><strong>Physical Activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>55 58.2±27.2</td>
<td>0.835</td>
<td>24 37.1±16.1</td>
<td>0.663</td>
<td>31 74.6±23.1</td>
<td>0.912</td>
</tr>
<tr>
<td>Yes</td>
<td>80 59.2±27.2</td>
<td></td>
<td>32 35.3±13.8</td>
<td></td>
<td>48 75.2±21.7</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol Consumption</strong></td>
<td></td>
<td>0.663</td>
<td></td>
<td>0.232</td>
<td>0.396</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>77 59.7±28.5</td>
<td></td>
<td>34 38.0±16.5</td>
<td></td>
<td>43 76.9±24.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>58 57.7±25.6</td>
<td></td>
<td>22 33.2±11.1</td>
<td></td>
<td>36 72.6±19.6</td>
<td></td>
</tr>
<tr>
<td><strong>Milk per day</strong></td>
<td>0.009</td>
<td>0.929</td>
<td>0.613</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>39 65.9±30.1</td>
<td></td>
<td>12 37.4±16.7</td>
<td>27 78.6±25.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; ½ pint</td>
<td>31 51.4±23.5</td>
<td></td>
<td>17 37.1±16.2</td>
<td>14 68.9±18.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>½ pint</td>
<td>38 65.0±26.5</td>
<td></td>
<td>9 33.5±11.7</td>
<td>29 74.7±21.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 pint</td>
<td>21 49.5±24.3</td>
<td></td>
<td>13 35.0±14.8</td>
<td>8 73.1±16.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1 pint</td>
<td>4 31.3±6.7</td>
<td></td>
<td>4 31.3±6.7</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Egg per week</strong></td>
<td>0.002</td>
<td>0.093</td>
<td>0.095</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>3 62.5±15.7</td>
<td></td>
<td>1 52.1</td>
<td>2 67.8±18.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than once</td>
<td>21 51.4±23.3</td>
<td></td>
<td>9 30.7±13.0</td>
<td>12 66.8±16.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>29 47.1±23.1</td>
<td></td>
<td>17 30.6±9.1</td>
<td>12 70.4±15.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-5 times</td>
<td>61 b 39.5±15.7</td>
<td></td>
<td>24 35.9±14.8</td>
<td>37 73.8±23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5 times</td>
<td>20 60.3±26.6</td>
<td></td>
<td>5 44.9±21.5</td>
<td>15 88.3±25.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>77.5±30.5 a</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oily fish per week</strong></td>
<td>0.044</td>
<td>0.039</td>
<td>0.140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>30 56.0±</td>
<td></td>
<td>10 31.9±12.1</td>
<td>20 68.1±17.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than once</td>
<td>58 23.3</td>
<td></td>
<td>17 31.6±16.2</td>
<td>41 78.4±23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>33 64.7±30.3</td>
<td></td>
<td>21 c 30.6±11.7</td>
<td>12 75.8±22.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-5 times</td>
<td>12 51.7±24.2</td>
<td></td>
<td>8 37.1±8.7</td>
<td>4 61.1±20.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5 times</td>
<td>1 52.7±20.8</td>
<td></td>
<td>0 48.4±21.2</td>
<td>1 109.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>109.7</td>
<td></td>
<td></td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Liver per week</strong></td>
<td>0.759</td>
<td>0.465</td>
<td>0.483</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>10 58.9±28.1</td>
<td></td>
<td>45 36.8±15.1</td>
<td>55 76.9±22.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than once</td>
<td>0 55.9±25.5</td>
<td></td>
<td>9 30.3±10.1</td>
<td>19 68.0±21.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Once</td>
<td>28 55.8±35.1</td>
<td></td>
<td>1 31.00</td>
<td>1 80.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-5 times</td>
<td>2 84.7</td>
<td></td>
<td>0 n/a</td>
<td>1 84.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 5 times</td>
<td>1 n/a</td>
<td></td>
<td>0 n/a</td>
<td>0 n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Supplement use (within the year)</strong></td>
<td>0.169</td>
<td>0.842</td>
<td>0.372</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>85 61.7±28.0</td>
<td></td>
<td>31 37.3±17.1</td>
<td>54 75.7±22.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>15 64.5±22.1</td>
<td></td>
<td>3 42.9±7.2</td>
<td>12 69.8±21.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish/fish liver oil</td>
<td>3 45.4±30.5</td>
<td></td>
<td>2 28.2±9.0</td>
<td>1 80.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish oil w/ vit D</td>
<td>14 55.2±22.6</td>
<td></td>
<td>5 30.4±5.3</td>
<td>9 69.0±15.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamins</td>
<td>13 41.9±25.2</td>
<td></td>
<td>12 35.9±14.0</td>
<td>1 113.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium w/ vit D</td>
<td>4 64.2±34.3</td>
<td></td>
<td>2 37.5±2.1</td>
<td>2 90.8±26.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values: mean ± SD
2 Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same column with same superscript letters are significantly different (²p = 0.014, ³0.001, ⁴0.034)
3.4.6.4 Sun exposure behaviour

Overall and within Brazil participants, there was a significant difference in serum 25(OH)D concentrations according to the amount body part exposed (both $p < 0.04$) as reported on the life-style questionnaire. Amongst Brazil participants, those reporting a recalled usual exposure of hands and face + arms and/or legs had significantly higher 25(OH)D concentrations than those exposing hands and face only ($78.5 \pm 21.8$ and $62.2 \pm 20.0$ nmol/l, respectively; $p = 0.029$) (Table 3.10).

There were no significant differences in 25(OH)D concentrations between sunscreen users and non-users. However, overall, those reporting using a SPF 15 sunscreen during holidays had significantly lower levels than those reporting the use of SPF of 40 or more ($34.1 \pm 16.6$ and $62.0 \pm 29.1$, respectively; $p = 0.034$). A post-hoc exploratory analysis, showed that amongst those reporting having the habit of sunbathing, 60% reported the use of SPF of 30, 40 or more during holidays compared to 9% using SPF 15 or 20 (n=43).
Table 3.10 The association between sun exposure behaviour and serum 25(OH)D concentrations in nmol/l, from lifestyle questionnaire

<table>
<thead>
<tr>
<th>Body parts exposed</th>
<th>All</th>
<th></th>
<th>England</th>
<th></th>
<th></th>
<th>Brazil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n 25(OH)D</td>
<td>p</td>
<td>n 25(OH)D</td>
<td>p</td>
<td>n 25(OH)D</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Face only</td>
<td>1 42.9</td>
<td>&lt;0.001</td>
<td>0.586</td>
<td>0.038</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hands and face</td>
<td>48 44.1 ±21.3</td>
<td></td>
<td>33 35.8 ±16.4</td>
<td>15 62.2 ±20.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hands/face + arms/legs</td>
<td>71 68.9 ±27.0</td>
<td></td>
<td>15 33.4 ±7.8</td>
<td>56 78.5 ±21.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hands/face + arms/legs + torso</td>
<td>15 59.4 ±25.2</td>
<td></td>
<td>7 42.2 ±18.2</td>
<td>8 74.2 ±21.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sunscreen use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>41 58.1 ±24.6</td>
<td>0.836</td>
<td>14 34.6 ±15.9</td>
<td>27 70.3 ±18.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>94 59.2 ±28.4</td>
<td></td>
<td>42 36.6 ±14.5</td>
<td>52 77.4 ±23.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPF at home§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>11 43.0 ±18.0</td>
<td>0.122</td>
<td>10 40.3 ±16.4</td>
<td>1 70.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1 54.9</td>
<td></td>
<td>0 0</td>
<td>1 54.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>37 64.9 ±27.3</td>
<td></td>
<td>13 38.3 ±13.3</td>
<td>24 79.4 ±21.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 or over</td>
<td>24 67.1 ±34.0</td>
<td></td>
<td>6 29.3 ±9.7</td>
<td>18 78.8 ±29.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPF at holidays§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>8 34.1 ±16.6</td>
<td>0.007</td>
<td>7 28.9 ±8.45</td>
<td>1 70.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>3 42.6 ±6.6</td>
<td></td>
<td>2 39.3 ±5.0</td>
<td>1 49.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>34 56.1 ±24.2</td>
<td></td>
<td>17 37.3 ±14.5</td>
<td>17 74.8 ±15.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 or over</td>
<td>39 62.0 ±29.1</td>
<td></td>
<td>15 36.6 ±14.7</td>
<td>24 77.9 ±24.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural sunbathing habit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43 59.5 ±26.9</td>
<td>0.667</td>
<td>33 34.9 ±12.9</td>
<td>59 73.3 ±20.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>92 57.4 ±28.2</td>
<td></td>
<td>23 37.9 ±17.1</td>
<td>20 79.9 ±20.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values: mean ± SD
2 Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same column with same superscript letters are significantly different (a p = 0.029 b p=0.034).

There were no significant associations between mean serum 25(OH)D between habitual body part exposed when outdoors in England and in Brazil reported on 90-days sun diaries (Table 3.11) (all p > 0.290), either overall or within each country. Serum 25(OH)D concentrations were significantly higher in those spending 15-30 minutes out in the sun compared to those spending less than 15 minutes (p=0.029).
3.4.6.5 Dietary Intake

Overall (n = 119), vitamin D and calcium intakes were statistically negatively correlated with 25(OH)D concentrations \( (r = -0.212, p = 0.021; r = -0.285, p = 0.002) \). The statistical significance was lost after controlling for individual sunlight exposure \( (r = -0.015, p = 0.875; r = -0.136, p = 0.160) \). Within each country, there were no significant correlations between vitamin D intake and 25(OH)D concentrations (all \( p > 0.117 \)).

Overall, there were no significant differences in mean vitamin D intake between vitamin D status groups, while calcium intakes were significantly lower \( (504.8 \pm 226.8 \text{ mg/d}) \) in women with levels above 75 nmol/l compared to those with levels below 25 and 50 nmol/l \( (836.6 \pm 449.2 \text{ mg/d}, p=0.007 \text{ and } 691 \pm 296.1 \text{ mg/d}, p=0.047, \text{ respectively}) \). In England residents mean vitamin D intake was significantly lower in women with vitamin D status 25 - 50 nmol/l compared to those with levels above 50 nmol/l \( (2.66 \pm 1.43 \text{ and } 4.29 \pm 2.97 \text{ µg/d}, p=0.040) \) (Table 3.12 and 3.13).
Table 3.12 Vitamin D status association with vitamin D mean dietary intakes

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th><strong>All</strong></th>
<th></th>
<th><strong>England</strong></th>
<th></th>
<th><strong>Brazil</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>p</td>
<td>n</td>
<td>Mean ± SD</td>
<td>p</td>
</tr>
<tr>
<td>&lt; 25 nmol/l</td>
<td>12</td>
<td>2.57 ±1.22</td>
<td>0.191</td>
<td>12</td>
<td>2.57 ±1.22</td>
<td>0.040</td>
</tr>
<tr>
<td>25-49.9 nmol/l</td>
<td>37</td>
<td>2.55 ±1.42</td>
<td></td>
<td>29</td>
<td>2.66 ±1.43</td>
<td></td>
</tr>
<tr>
<td>50-74.9 nmol/l</td>
<td>36</td>
<td>2.83 ±2.66</td>
<td></td>
<td>10</td>
<td>4.29 ±2.97</td>
<td></td>
</tr>
<tr>
<td>&gt; 75 nmol/l</td>
<td>34</td>
<td>1.91 ±1.53</td>
<td></td>
<td>0</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

1 Values: mean ± SD
2 Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same column with same superscript letters are significantly different (a p = 0.007; b p=0.047; c p=0.044).

Table 3.13 Vitamin D status association with calcium mean dietary intakes

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th><strong>All</strong></th>
<th></th>
<th><strong>England</strong></th>
<th></th>
<th><strong>Brazil</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>p</td>
<td>n</td>
<td>Mean ± SD</td>
<td>p</td>
</tr>
<tr>
<td>&lt; 25 nmol/l</td>
<td>12</td>
<td>836.6 ±449.2</td>
<td>0.005</td>
<td>12</td>
<td>836.6 ±449.2</td>
<td>0.305</td>
</tr>
<tr>
<td>25-49.9 nmol/l</td>
<td>37</td>
<td>690.1 ±296.1</td>
<td></td>
<td>29</td>
<td>671.8 ±256.5</td>
<td></td>
</tr>
<tr>
<td>50-74.9 nmol/l</td>
<td>36</td>
<td>600.3 ±297.9</td>
<td></td>
<td>10</td>
<td>762.9 ±292.8</td>
<td></td>
</tr>
<tr>
<td>&gt; 75 nmol/l</td>
<td>34</td>
<td>504.8 ±226.8</td>
<td></td>
<td>0</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

1 Values: mean ± SD
2 Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same column with same superscript letters are significantly different (a p = 0.007; b p=0.047; c p=0.044).

3.4.6.6 Individual UV radiation levels

Overall, daily individual UV radiation level showed a strong significant positive correlation with serum 25(OH)D concentrations (n=112, r = 0.661, p<0.001; n=3 outliers removed from analysis due to daily SED > 10) (Figure 3.10) and remained statistically significant after controlling for vitamin D3 intake (dietary intake), age and BMI (r = 0.669, p<0.001). Within
each country, there were no significant associations between daily UV radiation levels and 25(OH)D concentrations (p>0.05).

The scatterplot (Figure 3.10) shows the overall strong positive linear relationship between daily individual UV radiation levels and baseline serum 25(OH)D concentrations, (r= 0.673, p<0.001). In this linear model a daily exposure of 0.28 and 1.5 SED predicted a serum 25(OH)D concentration of 50 nmol/l and 75 nmol/l, respectively.

Figure 3.10 Relationship between baseline serum 25(OH)D concentration and baseline individual daily sunlight exposure level in participants with daily individual UVB exposure levels below 10 SED (n= 112)
Vitamin D status was associated with individual UV radiation (Table 3.14). Overall, women with serum 25(OH)D concentrations above 75 nmol/l had significantly higher mean UV radiation (2.26 ± 3.04 SED) than those with deficient (0.02 ± 0.01 SED), insufficient (0.25 ± 0.43 SED) and suboptimal (0.98 ± 1.00 SED) status (p<0.001).

Table 3.14 Vitamin D status association with mean individual UV radiation level

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>All n</th>
<th>Mean ± SD ²</th>
<th>p ¹</th>
<th>England n</th>
<th>Mean ± SD ²</th>
<th>p ¹</th>
<th>Brazil n</th>
<th>Mean ± SD ²</th>
<th>p ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 nmol/l</td>
<td>12</td>
<td>0.02 ± 0.01 a</td>
<td>&lt;0.001</td>
<td>12</td>
<td>0.02 ± 0.018</td>
<td>0.666</td>
<td>0</td>
<td>n/a</td>
<td>0.040</td>
</tr>
<tr>
<td>25 – 49.9 nmol/l</td>
<td>34</td>
<td>0.25 ± 0.43 b</td>
<td></td>
<td>26</td>
<td>0.038 ± 0.028</td>
<td>8</td>
<td>0.94 ± 0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 – 74.9 nmol/l</td>
<td>33</td>
<td>0.98 ± 1.00 c</td>
<td></td>
<td>8</td>
<td>0.037 ± 0.027</td>
<td>25</td>
<td>1.28 ± 0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 75 nmol/l</td>
<td>36</td>
<td>2.26 ± 3.04 a,b,c</td>
<td></td>
<td>0</td>
<td>n/a</td>
<td>36</td>
<td>2.26 ± 3.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Values: mean ± SD
²Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same column with same superscript letters are significantly different (a p=0.002; b p<0.001; c p=0.020).

To further explore the relationship between daily individual UV radiation and serum 25(OH)D concentration, a series of models were assessed for best fit and a non-linear cubic regression model was selected (n=112, R²= 0.0445, p<0.001) to investigate whether there is a threshold UV radiation level at which a plateau in serum 25(OH)D is evident. As shown in Figure 3.11, 25(OH)D concentrations seem to reach a maximum plateau around 75 - 85 nmol/l at daily UV radiation around 1.5 - 2.0 SED, based on the inflection point of the curve (i.e. the curve starts changing from being concave (concave upward) to convex (concave downward).
**Figure 3.11** Relationship between daily individual UV radiation and baseline serum 25(OH)D concentration (cubic model, \( p < 0.001 \)).

### 3.4.7 Prediction of circulating 25(OH)D concentrations: Mathematical Modelling

Preliminary analyses ensured no violation of normality, linearity, generalizability (sample size), multicollinearity and homoscedasticity.

Due to the differences in mean age and anthropometric measures and significant correlations with serum 25(OH)D, a hierarchical multiple regression was used to investigate the ability of daily individual UV radiation levels (SED) to predict 25(OH)D concentrations (nmol/l), after controlling for the influence of age and adiposity (Table 3.14). There were no significant correlations between vitamin D intake and serum 25(OH)D concentrations either overall or within each country after controlling for individual UV radiation level, and therefore vitamin D dietary intake was not included in this model.
Age and BMI were entered at Step 1, explaining 7.7% of the variance in 25(OH)D concentrations. After entry of daily individual sunlight exposure level at step 2, the total variance explained by the model as a whole was 46.5%. The added UV radiation measure explained an additional 38.8% of the variance in baseline 25(OH)D concentrations, after controlling for the influence of age and BMI, (F (3, 111) = 32.16, p<0.001). In the final model, only UV radiation made a unique statistically significant contribution (Beta= 0.654, p<0.001) in predicting 25(OH)D concentrations. According to the slope coefficient for daily individual UV radiation levels, 25(OH)D concentration increases by 20.2 nmol/l for each extra SED of UV radiation, regardless of age and BMI.

**Table 3.14** Hierarchical multiple regression of daily individual UV radiation levels (SED) ability to predict 25(OH)D concentration

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>R Square Change</th>
<th>F Change</th>
<th>Sig. F Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.277&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.077&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.060</td>
<td>26.44</td>
<td>0.077</td>
<td>4.65</td>
<td>0.011</td>
</tr>
<tr>
<td>2</td>
<td>0.682&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.465&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.451</td>
<td>20.22</td>
<td>0.388</td>
<td>80.57</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> Predictors: (Constant), age, BMI, waist circumference
<sup>b</sup> Predictors: (Constant), age, BMI, waist circumference, daily sunlight exposure
<sup>c</sup> Dependent Variable: Baseline serum 25(OH)D concentrations
Mean plasma PTH concentration in England residents was significantly higher than within Brazil residents (5.36 ±1.99 pmol/l and 4.49 ±1.47 pmol/l, respectively p=0.004). Plasma PTH concentrations ranged from 2.41 to 13.76 pmol/l within those living in England and from 2.18 to 8.51 pmol/l in participants living in Brazil.

PTH concentrations were negatively correlated with 25(OH)D concentrations (r= -0.285, p= 0.001). A series of regression analyses were conducted to model the relationship between serum 25(OH)D and PTH, and a non-linear regression cubic model was found to have the best fit (n = 134, R² = 0.113, p = 0.001), with PTH reaching a minimum plateau at 25(OH)D concentrations of around 70 - 80 nmol/l, based on the inflection point of the curve (i.e. the curve starts changing from being convex (concave upward) to concave (concave downward) (Figure 3.12).
Figure 3.12 Relationship between serum 25(OH)D and PTH concentrations (n=134; $R^2 = 0.113, p = 0.001$).

There was a statistically significant difference in mean baseline PTH concentrations between the different vitamin D status groups [$F(3, 130) = 6.5, p < 0.001; n=134$], with a large actual difference in mean PTH concentrations (effect size = 0.14). Post-hoc comparisons showed that mean PTH concentrations for deficiency ($6.60 \pm 2.47$ pmol/l) were significantly different from insufficient ($4.96 \pm 1.70$ pmol/l; $p=0.009$), adequate ($4.55 \pm 1.39$ pmol/l; $p=0.001$) and optimal ($4.42 \pm 1.51$ pmol/l; $p < 0.001$) status women (Figure 3.13).

** $p<0.01$; *** $p<0.001$ (ANOVA compared to [25(OH)D] < 25 nmol/l). Error bars represent SEM.

Figure 3.13 Differences in mean PTH concentrations by different vitamin D status (n=134).
3.4.9 Calcium concentrations

There were no significant differences in serum albumin-corrected calcium concentrations between England and Brazil residents (2.30 ± 0.07 and 2.28 ± 0.06 mmol/l respectively, \(p = 0.066\)), with all participants with concentrations within the normal range of 2.1 - 2.6 mmol/L.

3.4.10 Safety and adverse events

Only participants living in Brazil had serum 25(OH)D concentrations above 100 nmol/l (n=3), of which two women had baseline concentrations above 130 nmol/l (134.9 and 148.6 nmol/l). Serum calcium concentrations were within the reference range for all participants (< 2.5 mmol/l).
3.5 Discussion

The present analyses aimed to examine serum 25(OH)D concentrations in Brazilian women living in different latitudes, and potential influential factors in the studied populations within each country in order to account for the latitude difference. Furthermore, this study investigates a threshold serum 25(OH)D concentration where a plateau in plasma PTH is evident.

Altogether, 82.1% of these otherwise healthy Brazilian women living in England and 11.4% in Brazil had insufficient 25OHD concentrations (<50 nmol/l) at the beginning of winter. More worryingly, if the threshold of 75 nmol/l recommended by the Endocrine Society (72) for optimal levels is applied, suboptimal status was universal amongst England residents and affected half (49.3%) of the women in Brazil.

According to the 2001 UK Census, there were over 144,000 South Americans (defined by country of birth) living in England and Wales, with Brazilians representing by far the largest group (35%) (125). Although a higher prevalence of vitamin D deficiency in immigrant groups living in Northern latitudes in comparison to native Caucasian populations has been widely documented (58), the prevalence of vitamin D deficiency and insufficiency amongst a South American ethnic group living in England has not been demonstrated before in the literature. Moreover, recent studies show that with increasing duration of residence in the new country, immigrants’ health often worsens.

In this cohort, Brazilian women who had been living in England for more than 2 years had indeed lower 25(OH)D concentrations than their Brazilian peers who had been in England for less than that. The proportions found in this study for Southern England dwelling women are, in fact, similar to the prevalence in Caucasian adult women in the UK of 21.7% deficiency over the year, according to the latest SACN report (74). A recent 1-year prospective cohort study with South Asian (n = 35) and Caucasian (n = 105) women, aged 20-55 years, living in Surrey – the same location as for this study’s England trial – reported 10% of Caucasian and 80.8% of South Asian women having deficient levels, in the winter (58).

In contrast, amongst the women in this study living in Brazil, there were no records of levels below 25 nmol/l and therefore none of the participants were vitamin D deficient; 11.4%
presented insufficient levels (<50 nmol/l), 38% suboptimal levels (< 75 nmol/l) and half of them (50.6%) had levels above 75 nmol/l at baseline. A study conducted with 369 women of child-bearing age (21-49 years) in the country’s capital Brasília (15°S) – the same Mid-west region as this study’s Brazil trial, observed a prevalence of 32% insufficient (< 50 nmol/l) and 81% sub-optimal status (< 75 nmol/l), in summer/autumn (126). Another study, in the city of São Paulo (23°S), known for its high levels of pollution (127), reported a similar high prevalence of 38.45% insufficiency (< 50 nmol/l) and 75% suboptimal levels (< 75 nmol/l), amongst adults (men and women) during winter months (68). At an even closer to the equator latitude in Brazil, in Recife (8°S), with 894 men and women over 19 years of age, 28.5% and 43.5% had insufficient (<50 nmol/l) and sub-optimal (< 75 nmol/l) vitamin D status, respectively (128). Similarly, in a study that analysed a national sample of 11,247 adults in Australia (25°S) enrolled in a health programme in 1999/2000, mean serum 25(OH)D concentration was 63 nmol/l, the prevalence of vitamin D insufficiency (<50 nmol/l) was 31% and 73% had levels <75 nmol/l (129).

Although mean vitamin D and calcium intakes were higher amongst those living in the UK, the inadequate dietary Vitamin D intakes were universal in both countries, with all participants recording intakes well below the RDA recommendation of 15 μg/day. It is important to note that the differences in mean intake observed between the two countries could be due to differences in the food composition tables. Some foods may contain both vitamin D and 25(OH)D forms, the latter being more potent. The UK both account take the total dietary 25(OH)D amount when calculating vitamin D values (and therefore applying a conversion factor of 5), while the US and Brazil food composition tables estimates do not include 25(OH)D. Since there is no consensus regarding which conversion factors to use, the variation in conversion factor values might explain the differences observed in dietary intake between the two countries. Nevertheless, the findings from this study were fairly similar to the estimated intake in the UK adult population of 2-4 μg/d for ages 1.5 to 64 years (74). Although there are very few naturally rich foods, vitamin D dietary sources are far from negligible and can be particularly significant during winter months for ethnic groups living abroad, where cultural adaptation is required in many aspects.

For instance, it was shown in this cohort that women consuming eggs for more than 5 times a week had higher 25(OH)D concentrations compared to those eating once a week or less. Likewise, particularly more evident amongst those living in England, a more frequent
consumption of oily fish was associated with higher 25(OH)D concentrations. However, in this study there were no associations between vitamin D intake and serum 25(OH)D concentrations. Studies reporting a weak or not significant correlation between dietary vitamin D intake and serum 25(OH)D concentrations have suggested that vitamin D intake requirements to reach a given concentration of serum 25(OH)D may differ between individuals (24,130,131) and individual sunlight exposure might be a better predictor of 25(OH)D concentration than dietary vitamin D intake.

Previous studies have largely reported significant associations between vitamin D status and body composition (132,133). In this cohort overall, serum 25(OH)D concentration was significantly negatively correlated with age, weight, waist circumference and BMI, but statistical significance did not remain after controlling for individual UV radiation level and was not observed within each country. It might be that significant associations between vitamin D status and adiposity was not evident in these sample due to the majority of the women (61.5%) having a healthy BMI and therefore the impact of adiposity not being a strong factor as sunlight exposure for this population.

The influence of skin pigmentation has been well observed in studies showing poorer vitamin D status in dark-skinned individuals compared to white adults, with higher amounts of UVB required in pigmented skins to achieve a similar 25(OH)D concentrations as white skin. In the present study, no associations were found between 25(OH)D concentrations and self-declared ethno-race or skin type. The reason for this may be due to the potential inconsistency in self-declared ethno-race and skin colour in the Brazilian population because of subjective definitions and cultural influence on ethnic identification. In fact, this was indeed observed in this study where amongst the same group of people 63% identified themselves as white and 33.3% as brown while, conversely, 63% classified themselves as type III and IV (light and moderate brown) and 31.1% as type I and II (white). These findings suggest that simple and subjective classifications of ethnicity and skin colour to investigate the effect of skin pigmentation on vitamin D status might not be appropriate for some populations or countries. Other methods such as measures of melanin density via spectral reflectance of the skin (14) or classification by a trained researcher based on observed skin type characteristics might be better options in the Brazilian population.
Individual daily UV radiation levels were strongly positively correlated with serum vitamin D concentrations. Women with serum 25(OH)D concentrations above 75 nmol/l had remarkably higher mean UV radiation than those with deficient (< 25 nmol/l), insufficient (25.9 - 50 nmol/l) and suboptimal (50 – 74.9 nmol/l) status (p<0.001). Moreover, 38.8% of the total variance in 25(OH)D concentrations was explained uniquely by daily individual UV radiation, after controlling for the influence of age and BMI. It was estimated that in this cohort, each extra SED of UV radiation would increase 25(OH)D concentration by 20.2 nmol/l, independent of age and BMI.

A safe limit of daily UV radiation level enough to produce vitamin D has been previously reported to be 1 SED for skin types I-IV (74). This study predicted that daily individual UV radiation of around 0.5 SED would be sufficient to maintain serum 25(OH)D at 50 nmol/l and 1.5 SED for 75 nmol/l. Furthermore, serum 25(OH)D concentrations seem to reach a maximum plateau around 75 - 85 nmol/l at daily UV radiation over 1.5 - 2.0 SED. These findings are in accordance with the tightly auto-regulated vitamin D metabolism, which responds promptly to excessive endogenous production of vitamin D via exposure of the skin to sunlight. With continuous exposure to sun radiation, pre-vitamin D3 and vitamin D3 in the epidermis are degraded into biologically inactive photoproducts (1,7).

Amongst England dwelling D-SOL participants, 100% recorded daily exposure levels of less than 1 SED as well as around half of those living in Brazil. It is surprising that such a high proportion of participants in Brazil presented relatively low individual daily exposure levels, considering the still high minimum winter UV index in Brazil of 8.

The input from the 90-days sun diaries showed a different picture in comparison to the habitual body part exposed reported by participants on the lifestyle questionnaire. When reporting recalled habitual body part exposed when out in the sun, more than half (58.9%) of women in England reported exposing hands and face only, while in Brazil most women (70.9%) reported the habit of exposing hands and face plus arms and/or legs, with a statistically different proportion between the two countries (p<0.001). However, from daily input collected by the sun diaries there were no differences in either time spent outdoors or body part exposed between the two countries. Interestingly, in both countries around half of women reported exposure of hands and face only on most days and roughly the other half reported exposure of hands and face plus arms and/or legs. One explanation for the similar body part exposure
pattern observed in these two opposite countries might be that the higher UV irradiation and hotter climate leads to more sun protection via clothing coverage. The little time spent outdoors (majority in both countries spending less than 30 minutes per day out in the sun) supports the building evidence on the influence of modern urban living on vitamin D status. These contradictory findings suggest that sun exposure behaviour, particularly in sunny tropical countries, might involve factors that are more complex and requires more detailed questionnaires/sun diaries to better define the patterns in behaviour towards sunlight.

Furthermore, in Brazil specifically, 25(OH)D concentration was associated with recalled habitual body parts exposed, with higher concentrations amongst those reporting a usual exposure of hands and face + arms and/or legs compared to just hands and face, but no associations were observed from the data collected via the 90-days sun diary. Such observations build on the affirmation that individual UV radiation level is better determined by individual behaviour towards sunlight rather than estimated local UV radiation availability.

Additionally, although there were no differences in mean 25(OH)D concentrations between sunscreen users and non-users, those reporting using a SPF 15 sunscreen during holidays had significantly lower levels (nearly half the mean concentration) than those reporting the use of SPF of 40 or more. This may be a reflection of higher factor use being a marker for higher overall exposure and greater likelihood of sunbathing. In fact, it was observed in this study that amongst those reporting having the habit of sunbathing, 60% reported the use of SPF of 30, 40 or more during holidays compared to 9% using SPF 15 or 20. Such observations reinforce the importance of considering habitual behaviour towards sunlight and how it can affect 25(OH)D cutaneous production in the skin when determining vitamin D and sunlight exposure recommendations for different populations. From a holistic point of view, taking high factor sunscreen alone as an indicator of lower UV radiation reaching the skin, for instance, could potentially underestimate individual exposure UV radiation level if higher SPF is also a marker for greater length of time in the sun.

While the FAO/WHO suggested that the most efficient physiological approach to acquiring vitamin D for populations at the equatorial latitude range (42°N - 42°S) is through endogenous synthesis from sunlight exposure, their report did consider the contribution of dietary intake (from diet or supplements) to counteract the potential detrimental effects of excessive exposure to sunlight. The report specifically suggests that daily exposure of arms and face, without
sunscreen, for approximately 30 minutes would be enough to maintain adequate vitamin D levels. This is in accordance with the findings of this study of women living in Brazil and spending 15-30 minutes out in the sun having significantly higher levels of 25(OH)D concentrations than those spending less than 15 minutes per day outdoors. However, this amount of daily unprotected exposure to UV radiation in sunny locations can arguably increase the risk of skin cancer and aging effects on the skin, especially to fair skinned individuals.

To the author’s knowledge, this is the first study in the literature to directly compare individual sunlight exposure levels and habitual behaviour towards sunlight between opposite latitudes during wintertime (minimizing confounding from sun radiation intensity) within the same ethnic group (minimizing confounding due to cultural habits and skin pigmentation). Furthermore, up to this date, this is also the first study to measure habitual UV radiation levels using a personal UV dosimeter in Brazil and the first study to show the strong correlation of individual levels with vitamin D serum concentrations in the Brazilian population.

Although significant differences in serum vitamin D concentrations between the two countries were expected, it is no less remarkable that mean vitamin D concentrations of England residents were as low as 36 nmol/l compared to a mean 75 nmol/l in Brazil. Likewise, the difference in ranges between the two countries is still impressive: from 5.0 to 73.5 nmol/l within participants living in England and from 36.2 to 148.6 nmol/l within those living in Brazil. Similar serum concentrations to the observed in the present study in Brazil were observed in South Africa ranging from 70 to 170 nmol/l (134) and in individuals with reportedly high-UV exposure (tanners, surfers and outdoor workers) ranging between 25 – 162 nmol/l (89).

In summer months individuals would have adequate sunlight availability to produce vitamin D in England and potentially increase individual UV radiation, whereas individuals in Brazil would be exposed to a extremely high UV radiation and hotter climate, which could lead to changes in behaviour towards sunlight as well as individual UV radiation. For instance, a study conducted at latitude 23°S (São Paulo, Brazil) with 603 (118 men and 485 women) healthy volunteers aged 18-90 years, showed a significantly higher prevalence of individuals with vitamin D levels below 75 nmol/l (77.4%) in winter compared to the end of summer months (37.3%) (69). A longitudinal study in the UK, with Caucasian and (n = 88) South Asian women (mean (±SD); age 48.2 years (14.4)), showed that individuals with a higher seasonal change in 25(OH)D, adjusted for overall 25(OH)D concentration, showed increased levels of PTH,
suggest a possible detriment to bone health via increased levels of PTH in individuals with a larger seasonal change in 25(OH)D concentration. Further investigations with longitudinal study designs could provide further valuable information in regards to the seasonal cycling in 25(OH)D concentrations within each latitude and its effects on bone health, as well as maximum concentrations achieved over the year.

The definition of optimal status has been commonly derived from the level of 25OHD at which PTH levels reach a minimum plateau, i.e. the suppression of PTH secretion as a surrogate of optimal bone health, but this yields a wide range of estimates. Mean plasma PTH concentration in England residents was significantly higher than within Brazil residents, reflecting the significantly lower 25(OH)D concentrations in England dwelling women observed in this study. PTH concentrations were negatively correlated with 25(OH)D concentrations and PTH clearly reached a minimum plateau at 25(OH)D concentrations of 70 - 80 nmol/l, being of great relevance for discussions regarding the estimation of optimal cut-offs for vitamin D levels in the Brazilian population. Additionally, mean PTH concentrations for vitamin D deficient (< 25 nmol/l) status were significantly higher compared to insufficiency (25 – 49.9 nmol/l), adequate (50 – 74.9 nmol/l) and optimal (> 75 nmol/l) status, with a stronger statistical significance compared to the latter. Serum 25(OH)D concentrations at which PTH levels reach a minimum plateau vary substantially between studies, probably influenced by factors such as diet, sunlight exposure, methodology, age and sex. There is a growing consensus that serum 25(OH)D concentrations of at least 75-80 nmol/l are required for optimal bone health (135). These data taken together, suggests that 25(OH)D concentrations above 70 nmol/l are optimal to reduce stimulation of the parathyroid gland in Brazilian women.

Advisory agencies have consistently highlighted the challenges in establishing reference values for adequate vitamin D recommendations, particularly due to the differences in individual variation as well as the influence of environmental external factors. The inappropriateness of direct comparison of data from studies conducted in different locations is mainly due to significant variations in results between different laboratories, different latitudes and different populations / ethnic groups – and therefore influencing factors, adding greatly to the difficulty in finding a global consensus.

Another important limitation to the current recommendations regarding dietary intakes and sunlight exposure to maintain adequate levels is that they are generally based on studies with
mainly Caucasian populations in high latitude countries, with limited robust data for other ethnicity and different geographical locations. Consequently, there is a substantial lack of evidence on the effect of individual sunlight exposure in low latitude countries and their native non-Caucasian populations.

Although habitual exposure to sunlight has been long considered the most important determinant of adequate vitamin D status, still very few studies to this date have investigated the relationship between actual individual exposure to UV radiation and serum 25(OH)D concentrations, with most data derived from in vitro or animal studies (2,16,17). Additional evidence for this relationship comes largely from epidemiological studies in higher latitude countries, associating the prevalence of vitamin D inadequacy with latitude of residence or seasonal cycling of 25(OH)D concentrations, without actually measuring individual exposure (14, 15, 19).

This cross-sectional analysis addresses these key knowledge gaps with two parallel trials, using identical methodologies to examine same ethnicity and sex individuals, in opposite (high North vs. low South) latitudes. Moreover, all samples were analysed at the same high standard laboratory, at Imperial College London, UK. The D-SOL study has specifically examined the relative contribution of sunlight exposure on serum 25(OH)D concentrations.

The biggest contribution of the results here presented to the vitamin D field is the novel in vivo analysis confirming a strong and positive significant correlation between individual and habitual sunlight exposure and 25(OH)D concentrations, during wintertime, directly comparing countries located in opposite latitudes, and therefore with very different seasonal climatology. The present study also highlights the currently underappreciated variation in individual UV radiation level influenced by different behaviours towards sunlight exposure.

3.5.1 Strengths and limitations

The biggest strength of this cross-sectional analysis is the directly comparable data on serum 25(OH)D and PTH concentrations and daily individual UV radiation measurement that represents personal and habitual solar radiation in a real life scenario. Further strengths of the
present study include: serum 25(OH)D measurement via liquid chromatography–mass spectrometry, the gold-standard method for assessing vitamin D status and data collection during the same season in both countries. The sample can be considered representative of the younger (<50 years of age) adult Brazilian women population which included an appropriate range of socio-demographic characteristics.

Participants’ individual UV radiation exposure was measured with personal dosimeter badges worn for a 1-week period, building on the limited data on habitual individual radiation levels currently available. Additionally this study contributes valuable data to the current lack of studies on vitamin D status, sunlight exposure and influential factors in adult Brazilian women.

Limitations include data being restricted to healthy adult women, aged 20 to 59 years old, and might not reflect other populations such as men, children, adolescents and pregnant or older women. Participants on this study had a rather healthy BMI and therefore, findings may not reflect overweight and obese populations due to the known influence of adiposity in vitamin D status. These findings may also not be generalizable to other ethnic groups with different characteristics, habits or culture.

Questions on behaviour regarding sunlight exposure and physical activity could have been more detailed in order to better reflect the habitual and cultural aspects of the studied population (i.e. to distinguish between indoor and outdoor activities, habitual use of hats, preference for staying in the sun/shadow when outdoors, habit of going into outdoor swimming pool / open water swimming, perceptions towards tanning,). Some participants recorded never consuming milk but have also noted on their questionnaires consuming vegan alternatives for milk. This might have underestimated the consumption of milk in regards to vitamin D and calcium associations with 25(OH)D concentrations if they were choosing fortified versions.
Conclusions

In conclusion, this study has highlighted the strong association between vitamin D serum 25(OH)D concentrations and individual UV radiation. The prevalence of vitamin D deficiency and insufficiency was extremely high in adult Brazilian women residing in southern England. Moreover, no participants living in England and only half of participants living in Brazil had optimal vitamin D status. Vitamin D deficiency or inadequacy, strongly associated with low individual UV radiation levels, could put these women at a greater risk of poor bone health at the end winter, particularly in England.

When translating this into public health recommendations the data presented here suggests that current recommendation for the Brazilian adult population of 25(OH)D concentrations above 50 nmol/l might not be sufficient to reduce stimulation of the parathyroid gland in this population and concentrations above 70 nmol/l would instead be required for optimal status. Moreover, a daily individual UV radiation of around 1.5 SED would be predictive of a serum 25(OH)D concentration at 75 nmol/l, but a maximum plateau would be reached around 2.0 SED, meaning further exposure would not be beneficial and can pose a unnecessary potential for harmful outcomes.

Further work should focus on extending the sample to include a wider demographic range, including more overweight individuals, males and other age group, and assessing potential influencing factors in greater detail, both in Brazil itself and amongst Brazilian and Latin American ethnic groups living in the UK.
4 Vitamin D supplementation in high versus low latitudes: efficacy, necessity and interactions

4.1 Background

Although far from negligible, vitamin D dietary sources are limited and therefore may not be sufficient to compensate for inadequate cutaneous synthesis. The increasing prevalence of vitamin D insufficiency worldwide warrants efficient nutritional strategies to correct low vitamin D concentrations, particularly in individuals with probable limited cutaneous synthesis. Fortified foods and supplementation have been investigated over the past decade in search for an efficient strategy to prevent vitamin D deficiency in risk groups as well as in the general population.

There is still no consensus for a standard definition of the optimal serum levels of 25(OH)D which makes it difficult to critically interpret results from interventions with vitamin D supplementation. Consequently, it is still challenging to determine evidence-based recommendations for vitamin D supplementation intakes. Several studies have demonstrated the effect of vitamin D supplementation in raising serum 25(OH)D concentrations but these have included different doses, intervention regimens and populations, yielding variable results (10,83,136,137). Furthermore, the vast majority of randomized controlled trials of vitamin D supplementation have targeted white populations living in high latitude countries and mostly older participants (> 65 years).

The most common recommendations for vitamin D intake for the general adult (19 – 70 years old) population range from 10 µg/day (UK) (74) to 15 µg/day (US, Australia, Brazil) (54,71,138). With regards to supplementation strategies, it has been suggested that for healthy
individuals every 2.5 µg/day (100 IU/day) of supplemental vitamin D₃, increases serum 25(OH)D concentrations by approximately 1.7 to 2.5 nmol/l (0.7 to 1 ng/ml) (72). A systematic review including 76 trials with Caucasian individuals >50 years (n=6207) and vitamin D supplement doses ranging from 5 to 250 µg/d (median, 20 µg/d), reported an average increase in serum 25(OH)D concentrations of 1.9 nmol/l (0.8 ng/ml) per microgram of vitamin D₃ per day (139). However, it is important to note that several studies have also demonstrated that individuals with the lowest initial 25(OH)D concentrations present a greater response to supplementation (140).

Although many trials have investigated the effect of vitamin D supplementation on skeletal clinical outcomes, particularly fracture risk, few have been design to specifically investigate the efficacy of vitamin D supplementation on raising serum 25(OH)D levels depending on concomitant individual sunlight exposure levels, a fundamental factor to determining appropriate dietary recommendations. Consequently, public guidelines and policies should be adapted to the potentially different recommended vitamin D intakes necessary to achieve the optimal levels according to local ambient sunlight availability.

Because vitamin D status is a function of several individual and environmental factors, conclusions about the need and efficacy of vitamin D supplementation must be context and country specific. To date, the need and efficacy of vitamin D supplemental intake to increase and maintain serum 25(OH)D concentration above insufficiency (50 nmol/l) in low sunny latitudes are unclear and, likewise, little is known regarding the interaction between vitamin D supplementation and individual exposure to sunlight.
4.2 Objectives

- **Objective 1:**
  To compare the effectiveness of vitamin D supplementation of 15 μg/day compared to a placebo across opposite latitudes, in maintaining serum 25(OH)D concentrations above deficient and insufficient thresholds (25 and 50 nmol/l, respectively) in adult women.

- **Objective 2:**
  To explore the interaction between vitamin D supplementation, latitude and individual exposure to sunlight.

- **Objective 3:**
  To investigate the influence of baseline circulating 25(OH)D concentrations on post-intervention concentrations.

- **Objective 4:**
  To investigate the response of PTH concentrations to vitamin D supplementation of 15 μg/d compared to a placebo across opposite latitudes.

4.3 Methods

The study recruited participants at the University of Surrey (51°N), England, at the end of autumn 2016 and participants at the University of Goiás (16°S), Brazil, at the end of autumn 2017. All participants at commencement of the study provided a written informed consent. Full clinical and methodological study details, including participant recruitment, randomization and data collection, are described in Chapter 2.
4.3.1 Statistical Analysis

Statistical analysis of the data was undertaken using SPSS software for Windows (version 25.0; IBM Corp, Armonk, NY).

Data was tested for normal distribution using the Kolmogorov-Smirnov tests. Non-normally distributed variables were log transformed and reported in the original scale. Non-parametric tests were used when log transforming did not normalise the data.

Descriptive statistics were determined for all variables. Continuous variables were presented as mean ±SD for normally distributed variables or as median (25%, 75% percentiles) for not normally distributed. For categorical variables, frequency and percentage were reported.

The efficacy of intervention was tested by intention to treat (ITT) and per protocol (PP) analyses. There were no differences between the two analyses and therefore, only ITT analysis is reported in this chapter. For the ITT analysis, pairwise exclusion technique was used to handle missing data. (PP analysis details can be found in Appendix I)

Paired t-tests were used to examine response to vitamin D supplementation (change in serum 25(OH)D and plasma PTH concentrations from baseline to post-intervention) in the two intervention groups (placebo and 15 μg/ vitamin D) within each country of residence. One-way ANOVA with post-hoc Tukey’s test was used to analyse the differences in post-intervention serum 25(OH)D and plasma PTH concentrations and total change in 25(OH)D and PTH concentrations between the four intervention/country groups namely: placebo in England, 15 μg/d in England, placebo in Brazil, 15 μg/d in Brazil. An analysis of covariance (ANCOVA) was also performed to examine these differences but controlling for baseline 25(OH)D concentrations, age and BMI.

Chi squared tests were used to compare the distribution of serum 25(OH)D status, at baseline and post-intervention, according to the pre-defined cut-off thresholds between country and between intervention groups in each country.
Pearson’s correlation were used to analyse the relationship between baseline 25(OH)D concentrations, total intake and individual UV radiation and post-intervention total change in 25(OH)D concentrations.

Factorial ANOVA was used to explore the interaction (individual and joint effect) of latitude and intervention (supplemented/placebo) on post-intervention serum 25(OH)D and total change in 25(OH)D concentrations.

Standard linear and multiple regression models were run to investigate the predictive ability of total vitamin D intake (from diet and supplementation) and individual daily sunlight exposure on baseline and achieved serum 25(OH)D concentrations post wintertime (post-intervention). Preliminary analyses ensured no violation of normality, linearity, generalizability (sample size), multicollinearity and homoscedasticity.

Baseline serum 25(OH)D concentrations were also added to the model predicting achieved serum 25(OH)D concentrations to investigate the effect of initial serum 25(OH)D levels (baseline).

Due to the extremely large influence of initial serum 25(OH)D concentrations, a hierarchical multiple regression was chosen to assess the ability of total vitamin D intake (from diet and supplementation) and individual daily sunlight exposure to predict serum 25(OH)D concentrations achieved post-intervention, after controlling for the influence of initial serum 25(OH)D concentrations.

A p value of <0.05 was considered significant.
4.4 Results

4.4.1 Recruitment

A total of 335 adult Brazilian women were screened for the study, 148 in England and 187 in Brazil, of which: 131 were excluded based on the exclusion criteria; 53 decided not to participate after the screening process and 15 did not show for baseline visit (Figures 4.1 and 4.2). (Reasons for exclusion at screening are detailed in Figure 3.1 in Chapter 3).

After the screening process, 136 women attended the baseline visit (n = 56 in England and 80 in Brazil). Of those, 122 completed the 12 weeks intervention and attended the final visit. The majority of dropouts were due to unwillingness or difficulty in attending final visit (n = 12). Other than that, one participant did not complete the study due to illness not related to the study and for one participant laboratory results were not available.

The Intention To Treat analysis included all participants that had valid baseline data for serum 25(OH)D concentration and were randomized into one of the intervention groups (n=135). Subjects were excluded from the Per Protocol analysis (n = 95) due to: intervention compliance below 75% (n = 18), assessed by leftover tablets count; menopausal status (n = 5); no post-intervention blood sample for serum 250HD concentrations (n = 14).

Compliance was satisfactory, with 86% of all participants achieving at least 75% compliance (84%, 100%, 84% and 80% achieving 75% compliance in the England Placebo, England 15 μg/d, Brazil Placebo and Brazil 15 μg/d groups, respectively; \( p=0.123 \)). There were no significant difference in compliance between the placebo and supplemented groups within each country (\( p=0.10 \) for the England trial and \( p=0.67 \) for the Brazil trial).
Figure 4.1 CONSORT flow diagram of participant enrolment, randomisation and analysis by study intervention groups: England and Brazil Trial
4.4.2 Participant Characteristics

4.4.2.1 Age, Adiposity and Skin Pigmentation

The baseline anthropometric characteristics for study participants by intervention group for each country are shown in Tables 4.1. There were no significant differences for any of the anthropometric baseline characteristics between the two intervention groups (placebo/15 μg/day vitamin D) in either country. Brazilian women living in England were significantly older, heavier and had a greater waist circumference than those living in Brazil. There were no significant differences between Brazilian women living in England and in Brazil for skin type distribution.

Table 4.1 Baseline anthropometric characteristics of women living by intervention group for each country (n= 135)\(^1\)

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<tr>
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<th>England (n=56)</th>
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<tbody>
<tr>
<td></td>
<td>Placebo (n=29)</td>
<td>15 μg/day (n=27)</td>
<td></td>
<td>Placebo (n=39)</td>
<td>15 μg/day (n=40)</td>
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<tr>
<td>Age (years)</td>
<td>33 (29, 41)</td>
<td>33 (28, 43)</td>
<td>1.000</td>
<td>27 (24, 31)</td>
<td>26 (24, 30)</td>
<td>0.883</td>
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<td></td>
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<td></td>
<td>&lt;0.001</td>
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<tr>
<td>Weight (kg)</td>
<td>65.5</td>
<td>67.8</td>
<td>0.476</td>
<td>59.25</td>
<td>60.3</td>
<td>0.980</td>
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<td></td>
<td>(58.9, 71.3)</td>
<td>(61.7, 83.6)</td>
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<td>(53.0, 70.0)</td>
<td>(54.7, 72.5)</td>
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<tr>
<td>Waist Circumference (cm)</td>
<td>84.3</td>
<td>88.3</td>
<td>0.420</td>
<td>70.5</td>
<td>70.1</td>
<td>1.000</td>
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<td></td>
<td>(74.1, 93.0)</td>
<td>(80.0, 99.9)</td>
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<td>(65.2, 76.7)</td>
<td>(67.0, 78.3)</td>
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<tr>
<td>BMI (kg/cm(^2))</td>
<td>24.57</td>
<td>25.21</td>
<td>0.883</td>
<td>22.2</td>
<td>23.5</td>
<td>0.784</td>
<td>0.028</td>
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<td></td>
<td>(21.3, 27.9)</td>
<td>(23.3, 28.2)</td>
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<td>(20.2, 25.0)</td>
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<td>BMI category [n (%)](^R1)</td>
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<tr>
<td>Healthy</td>
<td>16 (55.1)</td>
<td>13 (48.1)</td>
<td>0.762</td>
<td>29 (74.4)</td>
<td>25 (62.5)</td>
<td>0.165(^a)</td>
<td>0.471(^a)</td>
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<tr>
<td>Overweight</td>
<td>8 (27.6)</td>
<td>8 (29.6)</td>
<td>6 (15.4)</td>
<td>10 (25)</td>
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<tr>
<td>Obese</td>
<td>5 (17.2)</td>
<td>6 (22.2)</td>
<td>4 (10.3)</td>
<td>5 (12.5)</td>
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<tr>
<td>Body fat (%)</td>
<td>30.3 ± 6.7</td>
<td>31.6 ± 4.7</td>
<td>0.384(^b)</td>
<td>37.2 ± 8.2</td>
<td>40.0 ± 8.6</td>
<td>0.149(^b)</td>
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<td>&lt;0.001(^c)</td>
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<tr>
<td>Skin type [n (%)](^R2)</td>
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<tr>
<td>Type I and II</td>
<td>8 (27.6)</td>
<td>9 (33.3)</td>
<td>0.365</td>
<td>13 (33.3)</td>
<td>23 (30)</td>
<td>0.946(^a)</td>
<td>0.875(^a)</td>
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<tr>
<td>Type III and IV</td>
<td>19 (65.5)</td>
<td>18 (66.7)</td>
<td>23 (59)</td>
<td>25 (62.5)</td>
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<tr>
<td>Type V and VI</td>
<td>2 (6.9)</td>
<td>0 (0)</td>
<td>3 (7.7)</td>
<td>3 (7.5)</td>
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<td></td>
</tr>
</tbody>
</table>

\(^1\) Values are median (25\(^{th}\), 75\(^{th}\) percentile), mean ± SD or n (%).

Statistical analysis: \(^2\) Between intervention group within each country: Mann-Whitney U test unless otherwise stated; \(^3\) Chi Squares, \(^4\) Independent t-test; \(^5\) Between the four intervention groups: Kruskall-Wallis test unless otherwise stated; \(^6\) Chi Squares; \(^7\) ANOVA \(^8\) Measurements derived from different methodologies (England: bioimpedance; Brazil: DXA scan). References: \(^R1\) (124); \(^R2\) (112)
4.4.2.2 Individual UV Radiation Levels

The average daily individual UV radiation levels, during wintertime, in week 0 (baseline) relative to week 12 (post-intervention) are shown in Figure 4.2. Values are expressed in units of standard erythema dose (SED).

At both baseline and week 12, median daily individual UV radiation level for those living in England was significantly lower (0.029 (0.015, 0.045) and 0.055 (0.028, 0.100) SED at baseline and week 12, respectively) than for those living in Brazil (0.934 (0.721, 1.790) and 1.197 (0.659, 1.861) SED, at baseline and week 12, respectively) ($p<0.001$). There were no significant differences for daily individual UV radiation level between intervention groups (placebo/15 μg/day vitamin D), for either country (Tables 4.2). Mean daily individual UV radiation for those living in England was significantly higher by the end of the trial compared to baseline period ($p=0.002$), while there were no significant differences between these two measurements for women living in Brazil ($p=0.991$).
A) Overall

Figure 4.2 Average daily individual UV radiation levels respective to week 0 (baseline) and week 12 (post-intervention), in units of standard erythema dose (SED), for the overall sample (A) and zoomed in to England participants (B).

1 8 subjects removed from this graphic representation due to daily levels above 4 SED in order to avoid disproportionate influence on the width of the scatter.
Table 4.2 Daily individual UV radiation level by country and intervention group

<table>
<thead>
<tr>
<th></th>
<th>England</th>
<th></th>
<th>Brazil</th>
<th></th>
<th>p²</th>
<th>p³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n=24)</td>
<td>15 μg/day (n=22)</td>
<td>Placebo (n=35)</td>
<td>15 μg/day (n=34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.028</td>
<td>(0.011, 0.054)</td>
<td>0.632</td>
<td>0.909</td>
<td>(0.735, 1.245)</td>
<td>0.418</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.050</td>
<td>(0.026, 0.104)</td>
<td>0.074</td>
<td>0.630</td>
<td>(0.032, 0.100)</td>
<td>0.630</td>
</tr>
</tbody>
</table>

1 Values are median (25th, 75th percentile).
Statistical analysis with log transformed data:
2 Between intervention group within each country: Independent t-test;
3 Between the four intervention groups: ANOVA

4.4.2.3 Dietary Vitamin D and Calcium Intakes

There were no significant differences in mean vitamin D or calcium intakes between the intervention groups (15 μg & placebo) at baseline in either country (p>0.05). Both vitamin D and calcium intakes were significantly higher in England groups compared to Brazil. (Table 4.3). By the end of intervention, in the England trial, mean vitamin D intake was significantly higher in the placebo group compared to the 15 μg/day group (3.4 ± 2.3 and 2.12 ± 1.18 μg/d, respectively; p=0.035). There were no significant differences between placebo and 15 μg/day group for vitamin D intake in Brazil nor for calcium intake in either country.

There were no significant differences between baseline and week 12 vitamin D intakes for any of the four intervention groups. Mean calcium intakes were significantly lower post-intervention in the England 15 μg/day group, and in both (placebo and 15 μg/day) Brazil groups (all p < 0.015), but there were no changes over time for the England placebo group. The difference in calcium intakes meant that 56%, 62.5%, 80.6% and 85.3% of participants did not meet the RNI reference of 700 mg/day at week 12 compared to 40.7%, 58.3%, 65.7% and 84.8% at baseline for England placebo, England 15 μg/day, Brazil placebo and Brazil 15 μg/day groups respectively. (Table 4.3).
Table 4.3 Vitamin D and calcium intakes by country and intervention group

<table>
<thead>
<tr>
<th>Intake / day</th>
<th>Vitamin D (μg)</th>
<th>Calcium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 12</td>
</tr>
<tr>
<td>Placebo (n=27)</td>
<td>2.5 (1.5, 4.7)</td>
<td>2.6 (1.4, 3.9)</td>
</tr>
<tr>
<td>15 μg/day (n=24)</td>
<td>2.2 (1.5, 5.1)</td>
<td>2.2 (1.1, 3.2)</td>
</tr>
<tr>
<td>Placebo (n=35)</td>
<td>1.3 (0.69, 2.81)</td>
<td>1.6 (0.8, 2.7)</td>
</tr>
<tr>
<td>15 μg/day (n=34)</td>
<td>1.4 (1.1, 2.6)</td>
<td>0.815</td>
</tr>
<tr>
<td>p⁴</td>
<td>0.076</td>
<td>0.620</td>
</tr>
</tbody>
</table>

| Baseline       | 713.6 (576.9, 876.9) | 595.4 (321.9, 866.1) | 0.108 |
| Week 12        | 676.9 (505.4, 889.1) | 566.9 (496.2, 776.1) | 0.342 |
| p⁴          | 0.141          | **0.001**     | **0.013** |

1 Values are median (25th, 75th percentile), Statistical analysis with log transformed data: ² Between intervention group within each country: Independent t-test; ³ Between the four intervention groups: ANOVA; ⁴ Between baseline and week 12: Paired t-test

4.4.3 Intervention outcomes

Per Protocol (PP) analysis did not change intervention outcomes, therefore only ITT is reported in this section. PP analysis can be found in Appendix I.

4.4.3.1 Response to vitamin D supplementation

There were no differences in serum 25(OH)D concentrations at baseline between the two intervention groups in either country (p>0.05).
Table 4.4 Serum 25OHD, plasma PTH and serum calcium concentrations at baseline and post-intervention in England and Brazil trials (n=135)\textsuperscript{1}

<table>
<thead>
<tr>
<th></th>
<th>England</th>
<th>Brazil</th>
<th>( p ) \textsuperscript{4}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>15 µg/day</td>
<td>Placebo</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>34.9 ±14.0</td>
<td>37.4 ±15.7</td>
<td>0.537</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>41.1 ±17.7</td>
<td>55.2 ±12.2</td>
<td>\textbf{0.002}</td>
</tr>
<tr>
<td>Total Change</td>
<td>5.3 ±14.9</td>
<td>17.0 ±17.1</td>
<td>\textbf{0.014}</td>
</tr>
<tr>
<td>( p ) \textsuperscript{5}</td>
<td>0.094</td>
<td>\textless 0.001</td>
<td>0.221</td>
</tr>
</tbody>
</table>

| Plasma PTH (pmol/l) |         |         |                     |             |
| Baseline            | 5.26 ±1.35 | 5.48 ±2.53 | 0.687 | 4.71 ±1.47 | 4.27 ±1.47 | 0.191 | \textbf{0.022} |
| Post-intervention   | 6.44 ±2.15 | 5.98 ±2.21 | 0.475 | 5.44 ±2.00 | 4.63 ±1.72 | 0.072 | \textbf{0.005} |
| Total Change        | 1.01 ±2.29 | 0.30 ±2.35 | 0.289 | 0.67 ±2.07 | 0.28 ±1.60 | 0.373 | 0.526 |
| \( p \) \textsuperscript{5} | \textbf{0.046} | 0.253 | \textbf{0.046} | 0.364 |

| Serum Calcium (mmol/l) |         |         |                     |             |
| Baseline              | 2.31 ±0.07 | 2.30 ±0.08 | 0.567 | 2.27 ±0.05 | 2.28 ±0.07 | 0.407 | 0.226 |
| Post-intervention     | 2.30 ±0.06 | 2.28 ±0.06 | 0.374 | 2.27 ±0.06 | 2.29 ±0.06 | 0.081 | 0.224 |
| Total Change          | -0.01 ±0.06 | -0.01 ±0.06 | 0.953 | -0.004 ±0.06 | 0.006 ±0.05 | 0.464 | 0.568 |
| \( p \) \textsuperscript{5} | 0.386 | 0.322 | 0.447 | 0.311 |

\textsuperscript{1} Values mean ± SD; analysis of intention to treat
\textsuperscript{2} Albumin corrected serum calcium concentrations
Statistical analysis: \textsuperscript{3} Log transformed data
By intervention group within each country: Independent t-test;
\textsuperscript{4} Between the four intervention groups: ANOVA.
\textsuperscript{5} Between baseline and week 12: Paired t-test

Post intervention, mean 25(OH)D in each group was 41.1±17.7 nmol/l for Placebo/England, 55.2±12.2 nmol/l for Supplemented/England, 71.9±22.1 nmol/l for Placebo/Brazil and 84.9±21.0 nmol/l for Supplemented/Brazil (Table 4.4). There was a significant effect of vitamin D supplementation on post-intervention serum 25(OH)D concentrations between the four intervention groups independent of baseline 25(OH)D concentrations, age and BMI (ANCOVA F(3,111) = 7.885, \( p \textless 0.001 \), partial eta squared= 0.176) (Figure 4.3). In this model, baseline concentrations explained 44.4\% (\( p \textless 0.001 \)), and intervention group 17.6\% (\( p \textless 0.001 \)) of the variance in 25(OH)D post-intervention concentrations and neither age or BMI had a significant contribution to the difference observed (both \textgreater 0.360) (ANCOVA).
Mean total change in serum 25(OH)D concentrations in the supplemented groups was 17.0±17.1 nmol/l (45% increase from mean baseline, \( p<0.001 \)) in England and 7.6 ± 23.0 nmol/l (10% increase from mean baseline, \( p=0.004 \)) in Brazil. There were no significant changes in either of the placebo groups (\( p=0.087 \) and \( p=0.221 \), England and Brazil trials respectively) (Table 4.4). When the factors associated with 25(OH)D concentrations were added into a hierarchical multiple regression, 27% of the change in 25(OH)D concentrations was explained, with only baseline concentrations and intervention (15 μg & placebo) statistically significant, and intervention recording a higher beta value (\( \beta: 0.319, p<0.001 \)) than baseline concentrations (\( \beta: -0.5, p<0.001 \)). Country of residence did not have a unique statistically significant contribution to the model, neither did age or BMI.
Differently from the placebo, vitamin D supplementation raised mean 25(OH)D concentrations from insufficiency (<50 nmol/l) to a mean concentration of 55.2 ±12.2 nmol/l in England and maintained mean concentration above adequacy (> 75 nmol/l) in Brazil (Figure 4.4). (A supplementary figure for individual response to intervention according to baseline serum 25(OH)D concentrations by intervention group and country can be found in Appendix I).

**Figure 4.4** Change in serum 25(OH)D concentration in adult Brazilian women over the 12 week trial by intervention and country of residence

4.4.3.2 Distribution of serum 25(OH)D concentrations by cut-off thresholds at baseline and post-intervention by intervention group

**Figure 4.5** illustrates the distribution of adult women with deficient (< 25 nmol/l), insufficient (25 – 49.9 nmol/l), adequate (50 – 74.9 nmol/l) and optimal (> 75 nmol/l) serum 25(OH)D concentrations by intervention group and country of residence, at baseline and post-
intervention. At baseline there was no difference in the proportion of participants within each of the selected serum 25(OH)D concentrations cut-off thresholds by intervention group in each country separately (p=0.549 for England; p=0.892 for Brazil). In contrast, post-intervention there were significant differences between the placebo and 15 μg/d in each country (p=0.002 in England; p=0.005 in Brazil) as well as between the four intervention groups (p<0.001).

Figure 4.5 Proportion of women with deficient (< 25 nmol/l), insufficient (25 – 49.9 nmol/l), sufficient (50 – 74.9 nmol/l) and optimal (> 75 nmol/l) serum 25(OH)D concentrations by intervention group at baseline and post-intervention.

Within women living in England, vitamin D supplementation prevented 25(OH)D concentrations below 30 nmol/l while nearly half (41.7%) in the placebo group had concentrations below this threshold. The 12-weeks supplementation clearly prevented vitamin D deficiency throughout winter in a high latitude location, with no supplemented women with post-intervention concentrations below 25 nmol/l compared to an 18.5% prevalence in this group at baseline. In the placebo group, 20.8% of women had post-intervention concentrations below 25 nmol/l compared to 31% at baseline. Furthermore, post-intervention 76% of the
supplemented group in England had a vitamin D status above the insufficiency threshold of 50 nmol/l compared to 18.5% at baseline, while in the placebo group only 25% had post-intervention levels above 50 nmol/l compared to 17.2% at baseline.

Post-intervention in Brazil, 75% of the women in the supplemented group had 25(OH)D concentrations above the optimal cut-off of 75 nmol/l compared to 50% at baseline. In the placebo group only 36.4% had levels above 75 nmol/l compared to 51.3% at baseline. Furthermore, only one participant (2.8%) in the supplemented group had insufficient levels below 50 nmol/l compared to 10% at baseline, while 9.1% of women in the placebo group had levels below this threshold compared to 12.8% at baseline.

**4.4.3.3 Effect of Baseline Serum 25(OH)D Concentration**

There were strong significant negative correlations between baseline serum 25(OH)D concentration and total change in serum 25(OH)D concentrations over the trial period, in both supplemented groups (England: $r = -0.728$, $p < 0.001$; Brazil: $r = -0.378$, $p = 0.021$) (Figures 4.6 and 4.7). This indicates that participants with the lowest baseline 25(OH)D concentrations had the greatest increase in response to vitamin D supplementation over the 12-week wintertime trial. The significant negative correlation remained in both supplemented groups after controlling for individual daily sunlight exposure at week 12, age and BMI ($r = -0.753$, $p < 0.001$ for England; $r = -0.464$, $p=0.01$ for Brazil).
A) Placebo

B) 15 μg vitamin D

Figure 4.6 Relationship between baseline serum 25OHD concentration and change in total serum 25OHD in the A) placebo and B) supplemented groups in England.
A) Placebo

![Graph showing relationship between baseline serum 25OHD concentration and change in total serum 25OHD in the A) placebo group.](image)

\[ r = -0.255 \]
\[ p = 0.152 \]

B) 15 μg vitamin D

![Graph showing relationship between baseline serum 25OHD concentration and change in total serum 25OHD in the B) supplemented group in Brazil.](image)

\[ r = -0.378 \]
\[ p = 0.021 \]

**Figure 4.7** Relationship between baseline serum 25OHD concentration and change in total serum 25OHD in the A) placebo and B) supplemented groups in Brazil.
4.4.3.4 Effect of daily individual UVB exposure

Overall, achieved serum 25(OH)D concentrations ranged from 19.5 to 84.7 nmol/l in England and from 31.3 to 141.5 nmol/l in Brazil. Considering supplementation combined with individual sunlight exposure levels, post-intervention half of the women receiving placebo and exposed to less than 1 SED were in the lowest quartile (58.1% below 50.4 nmol/l) of achieved serum 25(OH)D while half of the women vitamin D supplemented and exposed to 1 SED or over achieved the highest quartile (50% achieving over 63.7 nmol/l) (Table 4.5).

Table 4.5 Achieved quartiles of 25(OH)D at 12 weeks by intervention group and individual daily sunlight exposure level

<table>
<thead>
<tr>
<th>Achieved 25(OH)D at 12 weeks (nmol/l)</th>
<th>&lt; 1 SED</th>
<th>≥ 1 SED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>15 μg/d</td>
</tr>
<tr>
<td>Q1 (19.5 to 50.4) n= 26</td>
<td>18 (58.1%)</td>
<td>7 (21.2%)</td>
</tr>
<tr>
<td>Q2 (50.5 to 63.6) n= 25</td>
<td>5 (16.1%)</td>
<td>8 (24.4%)</td>
</tr>
<tr>
<td>Q3 (63.7 to 83.1) n= 25</td>
<td>4 (12.9%)</td>
<td>12 (36.3%)</td>
</tr>
<tr>
<td>Q4 (83.2 to 141.50) n= 25</td>
<td>4 (12.9%)</td>
<td>6 (18.1%)</td>
</tr>
</tbody>
</table>

Q = quartile; data presented as n (%) of participants in the intervention group achieving each quartile.

Overall, daily individual UV radiation levels, at either baseline or week 12, were not correlated with total change in serum 25(OH)D concentrations ($r = -0.062$, $p = 0.537$ and $r = -0.097$, $p = 0.318$) (Figures 4.8), even after controlling for baseline 25(OHD concentrations, age and BMI (all $p > 0.05$).
Figure 4.8 Relationship between daily individual UVB exposure levels at week 12 and change in total serum 25OHD (n=119)

4.4.3.5 Effect of total vitamin D intake

The relationship between achieved serum 25(OH)D concentrations and total vitamin D intake (diet plus supplemental vitamin D), in each latitude, in Brazilian adult women is shown in Figure 4.9. There was a significant positive correlation between total intake and achieved serum 25(OH)D concentrations in both countries, with a greater correlation coefficient for the England group (England: r = 0.443 p = 0.002; Brazil r = 0.269, p = 0.029).
A) England

![Graph showing relationship between achieved serum 25(OH)D concentrations and total vitamin D intake by country in England. The correlation coefficient is 0.443 with a p-value of 0.002.]

B) Brazil

![Graph showing relationship between achieved serum 25(OH)D concentrations and total vitamin D intake by country in Brazil. The correlation coefficient is 0.269 with a p-value of 0.029.]

1Mean response (central line) and 95% confidence intervals (coloured lines). Horizontal hashed lines represent serum 25(OH)D thresholds of 25, 50 and 75 nmol/l.

Note: Graphs in different scales for each country due to differences in range.

**Figure 4.9** Relationship between achieved serum 25(OH)D concentrations and total vitamin D intake by country
4.4.4 Vitamin D supplementation and latitude effects on serum 25(OH)D concentration: mathematical modelling

4.4.4.1 Main effects and interaction of vitamin D supplementation and latitude

A factorial ANOVA was used to compare the main effects of intervention (vitamin D supplementation or placebo) and latitude (low or high) and the interaction effect of vitamin D supplementation and latitude on achieved serum 25(OH)D concentrations, while controlling for baseline serum 25(OH)D concentrations.

The model showed a significant main effect of intervention (F(1,113) = 21.29, p<0.001) and explained 68.2% in the variance of achieved serum 25(OH)D concentrations (R² = 0.682). There was, however, no main effect of latitude (F(1,113) = 2.18, p = 1.42). There was also no significant interaction between the two factors (F(1,113) = <0.001, p = 0.996). This model confirms the ability of vitamin D supplementation of 15μg per day to raise serum 25(OH)D concentrations regardless of latitude. Adding age and BMI to the model did not change the reported outcomes, only increasing the R² to 0.684.

4.4.4.2 Inter-relationship amongst total vitamin D intake, baseline 25(OH)D concentrations and individual sunlight exposure in predicting achieved 25(OH)D concentrations – overall sample

Hierarchical multiple regression was used to assess the ability of vitamin D total intake (from diet and supplement) and individual sunlight exposure level at week 12 to predict achieved concentrations of serum 25(OH)D post wintertime (post-intervention) in the overall sample, after controlling for the influence of initial (baseline) serum 25(OH)D concentrations, country of residence, age, BMI and waist circumference (Table 4.6).

Serum baseline 25(OH)D concentration, country of residence, age, BMI and waist circumference were entered at Step 1, explaining 62.6% of the variance in achieved 25(OH)D concentrations. After entry of vitamin D total intake and individual sunlight exposure level at step 2, the total variance explained by the model as a whole was
67.5%. The two added measures explained an additional 4.9% of the variance in achieved 25(OH)D concentrations, after controlling for the influence of initial levels, country of residence, age, BMI and waist circumference, F (2, 99) = 7.393, p=0.001. In the final model, only baseline concentrations and total vitamin D intake made a unique statistically significant contribution: baseline 25(OH)D concentrations were the strongest predictor (Beta= 0.714, p<0.001) of post wintertime 25(OH)D concentrations explaining uniquely 24.4% of the variance, followed by total vitamin D intake explaining uniquely 4.8% of the variance (Beta= 0.226, p<0.001). Neither individual UVB exposure, country of residence, age, BMI or waist circumference made a unique contribution to the model.
Table 4.6 Hierarchical multivariate model of serum 25(OH)D: predictors of post wintertime 25(OH)D concentrations (n=119)

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>R Square Change</th>
<th>F</th>
<th>df1</th>
<th>df2</th>
<th>Sig. F Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.791(^a)</td>
<td>0.626</td>
<td>0.606</td>
<td>15.88656</td>
<td>0.626</td>
<td>31.815</td>
<td>5</td>
<td>95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.822(^b)</td>
<td>0.675</td>
<td>0.652</td>
<td>14.96983</td>
<td>0.049</td>
<td>6.996</td>
<td>2</td>
<td>93</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^a\) Predictors: (Constant), Serum 25OHD at baseline, country of residence, age, BMI or waist circumference  
\(^b\) Predictors: (Constant), Serum 25(OH)D at baseline, country of residence, age, BMI or waist circumference, total vitamin D intake, daily sunlight exposure  
\(^c\) Dependent Variable: Achieved serum 25(OH)D concentrations

Coefficients \(^a\)

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstand. Coefficients</th>
<th>Stand. Coefficients</th>
<th>95.0% Confidence Interval for B</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>t</td>
</tr>
<tr>
<td>1 (Constant)</td>
<td>33.98</td>
<td>17.14</td>
<td>1.982</td>
<td>0.050</td>
</tr>
<tr>
<td>Baseline 25OHD</td>
<td>0.687</td>
<td>0.083</td>
<td>0.740</td>
<td>8.301</td>
</tr>
<tr>
<td>Age</td>
<td>-0.097</td>
<td>0.206</td>
<td>-0.033</td>
<td>-0.468</td>
</tr>
<tr>
<td>BMI</td>
<td>0.969</td>
<td>0.692</td>
<td>0.193</td>
<td>1.400</td>
</tr>
<tr>
<td>Waist circ.</td>
<td>-0.370</td>
<td>0.284</td>
<td>-0.199</td>
<td>-1.301</td>
</tr>
<tr>
<td>Country</td>
<td>-0.085</td>
<td>5.649</td>
<td>-0.002</td>
<td>-0.015</td>
</tr>
</tbody>
</table>

ANOVA: F(5, 95) = 31.815, p<0.001

| 2 (Constant) | 27.84 | 16.44 | 1.693 | 0.094 | -4.813 | 60.49 | |
| Baseline 25OHD | 0.660 | 0.079 | 0.710 | 8.364 | < 0.001 | 0.503 | 0.816 | 0.655 | 0.494 |
| Age | -0.003 | 0.196 | -0.001 | -0.016 | 0.998 | -0.39 | 0.387 | -0.002 | -0.001 |
| BMI | 0.692 | 0.669 | 0.138 | 1.035 | 0.303 | -0.635 | 2.020 | 0.107 | 0.061 |
| Waist circ. | -0.35 | 0.269 | -0.189 | -1.301 | 0.197 | -0.886 | 0.185 | -0.134 | -0.077 |
| Country | 1.612 | 5.389 | 0.031 | 0.299 | 0.765 | -9.089 | 12.31 | 0.031 | 0.018 |
| Vitamin D intake | 0.754 | 0.203 | 0.225 | 3.714 | < 0.001 | 0.351 | 1.158 | 0.359 | 0.220 |
| Sun exposure | 0.175 | 0.590 | -0.019 | 0.297 | 0.767 | -0.996 | 1.346 | 0.031 | 0.018 |

ANOVA: F(7, 93) = 27.592, p<0.001

\(^a\) Dependent Variable: Achieved serum 25(OH)D concentrations
4.4.4.3 Inter-Relationship Amongst Total Vitamin D Intake, Baseline 25(OH)D Concentrations and Individual Sunlight Exposure in predicting achieved 25(OH)D concentrations – within each country

In each country separately, hierarchical multiple regressions models were used to assess the ability of vitamin D total intake (from diet and supplement) and individual sunlight exposure level at week 12 to predict achieved concentrations of serum 25(OH)D post wintertime (post-intervention), after controlling for the influence of initial (baseline) serum 25(OH)D concentrations. Age, BMI and waist circumference were not associated with 25(OH)D concentrations within each country sub-groups, and therefore not added to the following models.

In the England trial serum baseline 25(OH)D concentration (entered at Step 1) explained 19.4% of the variance in of achieved 25(OH)D concentrations. After entry of vitamin D total intake and individual sunlight exposure level at step 2, the total variance explained by the model as a whole was 34.3%. The two added measures explained an additional 14.8% of the variance in achieved 25(OH)D concentrations, after controlling for the influence of initial levels, F(3, 38) = 6.592, p=0.001. In the final model, only baseline concentrations (Beta= 0.385, p=0.007) and total vitamin D intake (Beta= 0.388, p=0.006) made a unique statistically significant contribution of 14% each, with similar strength. Individual UVB exposure did not make a unique contribution to the model (p = 0.976) (Table 4.7).
Table 4.7 Hierarchical multivariate model of serum 25(OH)D: predictors of post wintertime 25(OH)D concentrations in England (n=49)

<table>
<thead>
<tr>
<th>Model</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>R Square Change</th>
<th>F</th>
<th>df1</th>
<th>df2</th>
<th>Sig. F Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.440a</td>
<td>0.194</td>
<td>0.174</td>
<td>15.09923</td>
<td>0.194</td>
<td>9.622</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>0.585b</td>
<td>0.343</td>
<td>0.292</td>
<td>13.99321</td>
<td>0.148</td>
<td>4.287</td>
<td>2</td>
<td>38</td>
</tr>
</tbody>
</table>

a Predictors: (Constant), Serum 25OHD at baseline,
b Predictors: (Constant), Serum 25(OH)D at baseline, total vitamin D intake, daily sunlight exposure
c Dependent Variable: Achieved serum 25(OH)D concentrations

In the Brazil trial, serum baseline 25(OH)D concentration (entered at Step 1) explained 50.1% of the variance in achieved 25(OH)D concentrations. After entry of vitamin D total intake and individual sunlight exposure level at step 2, the total variance explained by the model as a whole was 54.9%. The two added measures explained an additional 6.3% of the variance in achieved 25(OH)D concentrations, after controlling for the influence of initial levels, ANOVA: F(3, 55) = 24.581, p<0.001. In the final model, only baseline concentrations and total vitamin D intake made a unique statistically significant contribution: baseline 25(OH)D concentrations were the strongest predictor (Beta= 0.705, p<0.001) of post wintertime 25(OH)D concentrations explaining uniquely 49% of the variance, followed by total vitamin D intake (Beta= 0.248, p=0.007) explaining uniquely 6% of the variance. Individual UVB exposure did not make a unique contribution to the model (p = 0.803) (Table 4.8).
Table 4.8 Hierarchical multivariate model of serum 25(OH)D: predictors of post wintertime 25(OH)D concentrations in Brazil (n=69)

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>R Square Change</th>
<th>F</th>
<th>df1</th>
<th>df2</th>
<th>Sig. F Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.714&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.510</td>
<td>0.501</td>
<td>16.08605</td>
<td>0.510</td>
<td>59.284</td>
<td>1</td>
<td>57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.757&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.573</td>
<td>0.549</td>
<td>15.28779</td>
<td>0.063</td>
<td>4.054</td>
<td>2</td>
<td>55</td>
<td>0.023</td>
</tr>
</tbody>
</table>

<sup>a</sup> Predictors: (Constant), Serum 25OHD at baseline, <br><sup>b</sup> Predictors: (Constant), Serum 25(OH)D at baseline, total vitamin D intake, daily sunlight exposure <br>Dependent Variable: Achieved serum 25(OH)D concentrations

### Coefficients<sup>a</sup>

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Confidence Interval for B</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>T</td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>23.564</td>
<td>7.459</td>
<td>3.159</td>
</tr>
<tr>
<td></td>
<td>Baseline 25OHD</td>
<td>0.735</td>
<td>0.095</td>
<td>0.714</td>
</tr>
<tr>
<td>ANOVA: F(1, 57) = 59.284, p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(Constant)</td>
<td>17.225</td>
<td>7.427</td>
<td>2.323</td>
</tr>
<tr>
<td></td>
<td>Baseline 25OHD</td>
<td>0.725</td>
<td>0.091</td>
<td>0.705</td>
</tr>
<tr>
<td></td>
<td>Vitamin D intake</td>
<td>0.729</td>
<td>0.261</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>Sun exposure</td>
<td>0.148</td>
<td>0.589</td>
<td>0.022</td>
</tr>
<tr>
<td>ANOVA: F(3, 55) = 24.581, p&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Dependent Variable: Achieved serum 25(OH)D concentrations.

#### 4.4.4.4 Estimated vitamin D dietary requirements by country

The relationship between total vitamin D intake (diet plus supplement from intervention) and achieved post-intervention serum 25(OH)D concentration in adult women was shown previously in Figure 4.9. To illustrate how recommendations might vary between the two countries investigated in this study, linear modelling of the total vitamin D intake and post-intervention serum 25(OH)D concentration was used to estimate vitamin D intakes that would maintain serum 25(OH)D concentrations above selected thresholds in each country (Table 4.9 and 4.10).
The response to total vitamin intake (diet plus supplemental vitamin D) was 1.002 nmol/l in England and 0.791 nmol/l in Brazil, for every 1 μg (40 IU) of vitamin D intake. The recommended EAR for women living in England would be 12 μg/d (480 IU) of vitamin D intake to meet the 50 nmol/l threshold and 37 μg (1490 IU) to meet the 75 nmol/l threshold by the end of winter (p=0.001). The corresponding values for women in Brazil are 0 μg (0 IU) for the 50 nmol/l threshold and 4.5 μg (180 IU) daily for the 75 nmol/l threshold (p= 0.029).

**Table 4.9** Linear regression model of serum 25(OH)D: total vitamin D intake as a of post wintertime 25(OH)D concentrations in England (n=49)

<table>
<thead>
<tr>
<th>Model</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>R Square Change</th>
<th>F Change</th>
<th>df1</th>
<th>df2</th>
<th>Sig. F Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.443a</td>
<td>0.196</td>
<td>0.179</td>
<td>15.05191</td>
<td>0.196</td>
<td>11.460</td>
<td>47</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*aDependent Variable: Achieved serum 25(OH)D concentrations

**Coefficients a**

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
<th>95.0% Conf. Interval for B</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>1</td>
<td>(Constant)</td>
<td>38.259</td>
<td>3.656</td>
<td>10.465</td>
<td>&lt;0.001</td>
<td>30.904</td>
</tr>
<tr>
<td></td>
<td>Vit D intake</td>
<td>1.002</td>
<td>0.296</td>
<td>0.443</td>
<td>3.385</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ANOVA: F(1, 47) = 11.460, p=0.001

*aDependent Variable: Achieved serum 25(OH)D concentrations.
**Table 4.10** Linear regression model of serum 25(OH)D: total vitamin D intake as a predictor of post wintertime 25(OH)D concentrations in Brazil (n=69)

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
<th>R Square Change</th>
<th>F Change</th>
<th>df1</th>
<th>df2</th>
<th>Sig. F Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.269a</td>
<td>0.072</td>
<td>0.058</td>
<td>22.10926</td>
<td>0.072</td>
<td>4.985</td>
<td>1</td>
<td>64</td>
<td>0.029</td>
</tr>
</tbody>
</table>

*aDependent Variable: Achieved serum 25(OH)D concentrations

**Coefficients**

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>95.0% Conf. Interval for B</th>
<th>Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Std. Error</td>
<td>Beta</td>
<td>t</td>
</tr>
<tr>
<td>1 (Constant)</td>
<td>71.379</td>
<td>5.256</td>
<td>16.772</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vit D intake</td>
<td>0.791</td>
<td>0.354</td>
<td>0.269</td>
<td>2.233</td>
</tr>
</tbody>
</table>

*aDependent Variable: Achieved serum 25(OH)D concentrations.

**4.4.5 Plasma parathyroid hormone concentrations**

At baseline, mean PTH concentration in England residents was significantly higher than within Brazil residents (5.36 ±1.99 pmol/l and 4.49 ±1.47 pmol/l, respectively $p=0.004$). Baseline PTH concentrations ranged from 2.41 to 13.76 pmol/l within those living in England and from 2.18 to 8.51 pmol/l in participants living in Brazil.

There were no significant differences in plasma PTH concentrations at baseline between the placebo and intervention groups in either country (Table 4.4).

Post-intervention, there was a significant increase in PTH in the placebo groups, in both England and Brazil residents, over the 12-week trials (England residents: 1.01 ± 2.2 pmol/l, 19.2% increase, $p=0.04$; Brazil residents: 0.67 pmol/l, 14.2% increase, $p=0.04$) (Figure 4.10). Plasma PTH concentrations did not change significantly during the intervention period in either vitamin D3 supplemented group ($p=0.253$ and $p=0.149$ for women living in England and in Brazil, respectively, Table 4.4).
There were no significant differences at either baseline or post-intervention in serum corrected calcium concentrations between the intervention groups in each country (Table 4.4). There were also no significant changes within intervention groups post-intervention ($p>0.05$) (Table 4.4).

4.4.7 Safety and adverse events

Only participants living in Brazil had serum 25(OH)D concentrations above 100 nmol/l, either at baseline or post-intervention. Of those, three women in the placebo group (of which two already had baseline concentrations $> 100$ nmol/l) and four in the supplemented group (all with initial concentrations below 100 nmol/l) had post-
intervention concentrations between 100 – 120 nmol/l. One woman in the placebo group had baseline and post-intervention concentrations of 134.9 and 135.4 nmol/l respectively; and two women in the supplemented group had post-intervention concentrations of 140.9 and 141.5 nmol/l (with baseline concentrations of 148.6 and 70.6 nmol/l respectively).

Three participants (one in the England placebo, one in the England supplemented and one in the Brazil placebo group) had post-intervention plasma PTH concentrations above 10 pmol/l, of which two (both England dwelling) had 25(OH)D concentrations below 30 nmol/l and one (Brazil dwelling) above 80 nmol/l.

None of the participants, including those with such high 25(OH)D and PTH concentrations, reported any adverse events throughout the duration of the study. Serum calcium concentrations were within the reference range for all participants (< 2.5 mmol/l).
4.5 Discussion

In the present study, vitamin D supplementation of 15 μg/day is shown to be significantly effective in raising serum 25(OH)D concentrations, regardless of latitude. Post-intervention mean serum vitamin D concentrations increased significantly in the supplemented group while remaining unchanged in the placebo group, in both countries. Supplementation over winter prevented vitamin D deficiency (< 25 nmol/l) and maintained mean 25(OH)D concentrations above the insufficiency threshold (50 nmol/l) in England and maintained mean 25 (OH)D concentration above the optimal threshold (> 75 nmol/l), with 75% of women presenting optimal levels, in Brazil. In comparison to the supplemented groups, post-intervention 20.8% of the women in the England trial placebo group presented deficient levels and two thirds (63.6%) of the women in Brazil had suboptimal levels. Furthermore, vitamin D supplementation of a low dose (15 μg/d) over 12 weeks was shown to be significantly beneficial to bone health, regardless of latitude, evidenced by a significant increase in plasma PTH in the placebo groups whilst supplementation prevented this seasonal increase in both countries.

Although habitual exposure to sunlight has been long considered the most important determinant of adequate vitamin D status, few robust RCTs to this data have investigated the relative contribution of vitamin D dietary intake and photochemical production in the skin on serum 25(OH)D concentrations. Furthermore, advisory agencies have consistently highlighted the challenges in establishing reference values for adequate vitamin D status and dietary recommendations, particularly due to the differences in individual variation as well as the influence of environmental external factors. The unsuitability of direct comparison of data from studies conducted in different locations is mainly due to significant variations in results between different laboratories, different latitudes and different populations / ethnic groups – and therefore influencing factors, adding greatly to the difficulty in finding a global consensus.

An important limitation to the current recommendations regarding dietary intakes and sunlight exposure to maintain adequate levels is that they are mostly based on RCT’s with Caucasian populations in high latitude countries, with limited robust data for other
ethnicity and different geographical locations. Consequently, there is a substantial lack of evidence on the effectiveness and even necessity of vitamin D supplementation as well as the effect of individual sunlight exposure in low latitude countries and their native non-Caucasian populations.

In this study, the above-described challenges and limitations are addressed with two parallel randomized placebo-controlled trials, using identical methodologies to examine same ethnicity and sex individuals, in opposite (high North vs. low South) latitudes, to shed light on the current controversies surrounding vitamin D recommendations. Moreover, all samples were analysed at the same high standard laboratory, at Imperial College London, UK. The D-SOL study has specifically examined the relative contribution of vitamin D supplemental intake and sunlight exposure on serum 25(OH)D concentrations and the efficacy of vitamin D supplementation in raising and maintaining adequate vitamin D serum levels throughout winter, concomitantly preventing increase in PTH concentrations, regardless of latitude.

It has been suggested that the average adult would require initial treatment with up to 1,250 μg (50,000 IU) of vitamin D orally once per week for 6–8 weeks followed by 20-25 μg (800 - 1000 IU) orally thereafter to improve nutritional insufficiency (< 50 nmol/l) and 800 to 1000 IU of vitamin D daily to improve suboptimal levels (50-75 nmol/l) (141). A study with healthy white and African American men and women aged 18–65 years in Long Island, US (40°N) proposed a dose of 95 μg/d (3800 IU) for those above a 25(OH)D threshold of 55 nmol/l and a dose of 125 μg/d (5000 IU) for those below that threshold to achieve concentrations above 75 nmol/l (142). Another study in a high latitude location, in Ireland (United Kingdom), a dose-response RCT conducted with young adults (20-40 years) during winter estimated an increase of 1.96 nmol/l per 1 μg intake of vitamin D (143).

Few studies have estimated the intake of vitamin D required to increased 25(OH)D concentrations in Brazil, with most studies with institutionalized elderly. For instance a study conducted in the city of São Paulo (23°S) estimated that supplementation with
175 μg (7,000 IU) per day would increase 25(OH)D concentrations an average of 22.5 nmol/l after three months with this elevation achieving a plateau after six weeks (n=42) (144). In a similar elderly institutionalized population (n=46), a RCT estimated an average dose of 92.5 μg (3,700 IU) per day of vitamin D3 for six months to achieve concentrations of 86.5 nmol/l (145). However, these studies cannot be generalized to the wider adult population as there are many factors amongst institutionalized individuals, and particularly the elderly, that will not be the same for otherwise healthy adults, the main factor being probable likely variation in individual sunlight exposure. Up to this date, there are no published studies investigating vitamin D supplementation in healthy adult women in Brazil.

The Brazilian Society of Endocrinology and Metabology recommends as a practical rule that for every 1 μg (40 IU) supplemented it can be expected to achieve a 0.7 – 1.0 nmol/l increase in 25(OH)D concentrations, based on recommendations from US studies (138). In the present study, every 1 μg (40 IU) of vitamin D intake predicted an increase of 1.002 nmol/l in Brazilian women living in England and 0.791 nmol/l in Brazilian women living in Brazil. As shown in this chapter, total change in serum 25(OH)D is strongly influenced by initial concentrations, which explains the smaller increase in serum 25(OH)D concentrations from vitamin D supplementation in women living in Brazil, that had significantly higher baseline concentrations then those in England. This study also showed that women living in Brazil may not need an intake of 15 μg (600 IU) as currently recommended as an EAR of 4.5 μg (180 IU) daily was estimated to be enough to meet the 75 nmol/l threshold during winter months for 50% of the population, as per analysis based on the mean. On the other hand, it was estimated that Brazilian women living in England would need a daily EAR of 480 IU (12 μg) of vitamin D intake to meet the 50 nmol/l threshold and a daily EAR of 37 μg (1490 IU) to meet the 75 nmol/l threshold during winter (144).

From the regression analyses in the overall sample, 67.5% of the variance in 25(OH)D concentrations was explained by baseline serum 25(OH)D concentration, total vitamin D intake, and individual sunlight exposure level altogether. This represents a large predictive ability of these variables. Worth noting however, is that within each country baseline concentration was a remarkably stronger predictor in Brazil, explaining uniquely 49% of the variance in 25(OH)D concentrations while in England baseline
concentration contributed (explained uniquely 14%) as much to the variance in 25(OH)D concentrations as total vitamin D intake from diet and supplement (explained uniquely 14%). Sunlight exposure did not have a unique contribution to any of the models.

In fact, the total change in serum 25(OH)D concentrations from supplementation was negatively correlated with initial concentrations, meaning that participants with the lowest baseline 25(OH)D concentrations had the greatest increase in response to vitamin D supplementation over the 12-week wintertime trial. This is in accordance with several published studies that have reported a greater effect of vitamin D supplementation or stronger associations between serum 25(OH)D concentrations and health outcomes in individuals with lower baseline concentrations (139, 151).

Exposure to sunlight is often said to be the most important determinant of adequate vitamin D levels (1, 14, 15, 31). Although individual sunlight exposure did not have a unique contribution to this analysis, mean baseline 25(OH)D concentrations in women living in Brazil was considerably high at the optimal threshold of 75 nmol/l. In low latitude countries, most people would easily achieve the daily minimum 1.0 – 1.5 SED for vitamin D production, as previously reported in Chapter 3. It might be that for such locations, where UV irradiation is very high throughout the year, a minimum sun exposure dose is associated with higher levels but an increased sun exposure is not as relevant to raising vitamin D levels as assumed, due to 25(OH)D concentrations reaching a maximum plateau from sunlight exposure, as also shown in Chapter 3 of this Thesis.

Therefore, recommendations for increasing sun exposure time in such locations could needlessly expose individuals to a greater risk of detrimental overexposure, without having significant contribution to raising or maintaining vitamin D levels. Besides, because most people are likely to achieve the minimum exposure required, inadequate levels in some individuals in sunny countries might be better explained by other factors, and such individuals are likely to benefit more from recommending a higher vitamin D intake, either from diet or supplements, than changing sunlight exposure behaviour.
A few participants had baseline and/or achieved serum vitamin D concentrations above 120 nmol/l but no adverse events or symptoms were reported, supporting the safety of both 15 μg daily doses, regardless of latitude or initial concentrations, as well as higher circulating levels.

4.5.1 Strengths and limitations

The strengths of this study are in its parallel directly comparable placebo-controlled trials, designed specifically to investigate the contribution of both vitamin D sources, food and endogenous production via sunlight exposure, on serum vitamin D concentrations. Conducting both trials in each country’s respective wintertime lessened extreme disparities in UV radiation between high and low latitudes. The study had a satisfactory intervention compliance and drop-out rates were low. Serum 25(OH)D concentrations from both trials were measured at the same laboratory in the UK and using the gold standard LC-MS/MS technique. Participants’ individual actual UV radiation exposure was measured at two time-points, building on the limited data on individual exposure levels currently available. Additionally this study contributes valuable data to the current lack of studies on vitamin D status, supplementation and sunlight exposure in adult Brazilian women. Supplement dose for intervention matches the current recommendation for the studied population, being therefore translatable to public health strategies.

Limitations include the sample of Brazilian healthy adult women which may not be generalizable to other ethnic groups, age ranges, or people with certain health issues or males. A larger sample within each latitude might have given more power to better determine influential factors relationships and interactions with vitamin D status. A longer duration of the study with additional mid-point measurements could have potentially giving further relevant information on the interaction between response to supplementation and any seasonal variations in vitamin D levels. There was also a lack of opportunity to generate a dose-response data that would have provided valuable insight into the response to vitamin D and thus better elucidate the interactions being investigated in the study.
Conclusions

In conclusion, the D-SOL study parallel RCTs have demonstrated that a low dose vitamin D supplementation is a remarkably effective strategy in raising circulating 25(OH)D levels and prevented vitamin D deficiency in a high latitude and maintaining optimal status in a low latitude location, over winter months.

The results presented in this chapter suggest that those with the lowest baseline 25(OH)D concentrations had the greatest increase in response to vitamin D supplementation over the 12-week wintertime trial. Sunlight exposure did not contribute independently to increasing vitamin D serum concentrations. Therefore, recommendations of increasing individual exposure to sunlight are not likely to be an effective approach to significantly changing circulating 25(OH)D concentrations, during wintertime. In a low latitude location, those presenting lower levels are in fact in the same high UV radiation ambient as their vitamin D sufficient native fellows, and therefore are more likely to be affected by other influential factors on vitamin D status. For those in high latitude locations, from October to March there is nearly no UVB radiation and therefore increased exposure to sunlight will not be effective in producing vitamin D in the skin. The findings also suggest that the recommended vitamin D intake of 15 μg (600 IU), particularly as a supplement, is an adequate recommendation and indeed required throughout winter for optimal bone health in Brazilian women living at high latitude.

More importantly, it is evident from the findings reported in this thesis, that importance of recommendations for sunlight exposure and dietary intake for optimal vitamin D status elaborate specifically to Brazil. This is particularly relevant since the findings here presented indicate that current recommendation vitamin D intake based on US guidelines might be higher than the actual need for adult women living in this low latitude country.
5.1 Background

Calcium and vitamin D have been long established as essential nutrients with a major role in bone health. Calcium is the most abundant mineral in the body and vital to functions related to vascular contraction, muscle function, nerve transmission, intracellular signaling and hormonal secretion (146,147). Ninety nine percent of the body’s calcium supply is stored in the bones and teeth where it supports structure and function. Bone acts as the calcium reservoir to maintain constant calcium homeostasis, with around 99% of the organism’s calcium supply stored in the bones and teeth. Vitamin D also plays a key role in calcium homeostasis, promoting calcium absorption in the gut and resorption in the kidney, as well as stimulating bone formation and remodeling (146). Therefore, optimal intakes of calcium and vitamin D are generally regarded as fundamental factors in the prevention and treatment of osteoporosis.

Calcium can be found naturally in foods such as milk, dairy and some vegetables (i.e. kale and broccoli), as well as in fortified foods, being also available as a dietary supplement and as a component of some medicines (such as antacids)(71). The EAR (IOM, US) for calcium from all sources, or the daily intake level estimated to meet the calcium requirement for 97.5% of the population, is 1,000 mg/day for men ≤70 years and women ≤50 years, and 1,200 mg/day for men >70 years and women >50 years whereas the EAR, requirement to cover 50% of the population, is 800 mg/day for men ≤70 years and women ≤50 years, and 1,000 mg/day for men >70 years and women >50 years (148). In the UK the equivalent value is the Recommended Nutrient Intake (RNI) of 700 mg/day for men and women > 19 years old (149).

The two forms of vitamin D, ergocalciferol (vitamin D2) and cholecalciferol (D3) are found in few foods, such as oily fish, mushrooms and in smaller amounts in animal
origin foods including milk, eggs and meat (70). Vitamin D fortified foods and supplements are additional sources of vitamin D and intakes from these sources can vary substantially by product and country. The IOM recommends a dietary allowance (RDA) of 15 μg (600 IU) of vitamin D per day, with no distinction between children >1 year old and adults up to the age of 70 years and 800 IU for ages >70 years, whereas the SACN latest guidance report has recommended a daily intake of 10 μg (400 IU) for all adults and children > 1 year old for musculoskeletal health benefits (74,148).

The associations of calcium intake, vitamin D intake and status with bone mineral density (BMD) and the risk of fractures have been studied extensively over the past decades (137,150,151). A considerable amount of human evidence supports the well-recognized and intuitive contribution of calcium for bone homeostatic regulation and treatment strategies for osteoporosis. Observational studies as well as randomized controlled trials have generally shown that higher calcium and vitamin D intakes (either from food or supplementation), compared to lower, in the long-term may have relevant benefits to bone health and reduce the risk of osteoporosis by slowing the rate of bone loss associated with aging. (146,152–154).

Parathyroid hormone (PTH), a hormone secreted by the parathyroid glands in response to low serum calcium levels, is also well recognized as a fundamental part of bone homeostasis. PTH triggers the hydroxylation of 25(OH)D to the active form 1,25(OH)2D leading to enhanced intestinal absorption of calcium. Chronic elevated PTH concentrations can have significant negative impacts on BMD and consequently and increased risk of fractures over time (146,147,155). Low dietary calcium and vitamin D intakes, as well as inadequate vitamin D status, can be independent contributors to high PTH concentrations (8,43,50). The negative correlation between PTH and vitamin D, and negative effects of higher PTH on bone health, have been previously demonstrated but mostly in elderly and/or osteoporotic populations (50,108,156–158), and therefore there is still limited data on healthy adults, particularly in Latin America and Brazil (20).
5.2 Objectives

- **Objective 1**
  To analyse the influence of habitual dietary vitamin D and calcium intakes on bone health parameters assessed by either pQCT or DXA in adult women.

- **Objective 2**
  To examine the associations of serum 25(OH)D and plasma PTH concentrations with bone health parameters assessed by either pQCT or DXA in adult women.

5.3 Methods

All participants at commencement of the study provided a written informed consent. Full clinical and methodological study details are described in Chapter 2.

This is a cross-sectional analysis of endocrine status, i.e. 25(OH)D and PTH concentrations, calcium and vitamin D intake and bone mineral density in healthy adult Brazilian women in living England (51°N) and adult Brazilian women living in Brazil (16°S), recruited for the D-SOL study. The participants were screened according to exclusion criteria that included potential cofounders likely to affect vitamin D metabolism and pregnant or planning a pregnancy during the study period, as detailed previously in Chapter 2 of this thesis. All participants at commencement of the study provided a written informed consent.

For the England trial, a peripheral quantitative computed tomography (pQCT) was performed on the participant’s non-dominant forearm at the baseline visit only, to measure volumetric bone mineral density at the 4% and 66% radial site. For participants in Brazil, whole body bone mineral density and bone mineral content and lower spine and femur bone mineral density were measured via a DXA (dual energy X-ray absorptiometry) scan at baseline only. The same cut-off values for T-score classification (i.e., T-score ≥−1.0 defined as low-risk score, T-score between −1.0 and −2.5 defined as moderate, and ≤−2.5 defined as high-risk score for osteoporosis) (159) were applied for pQCT and DXA scans.
5.3.1 Statistical analysis

Statistical analysis of the data was undertaken using SPSS software for Windows (version 25.0; IBM Corp, Armonk, NY). For this cross-sectional analysis, menopausal women (n=5) were excluded due to the known effects of menopause on bone metabolism (160,161).

Data were tested for normal distribution using the Kolmogorov-Smirnov tests. Non-normally distributed variables were log transformed and reported in the original scale. Non-parametric tests were used when log transforming did not normalise the data. Descriptive statistics were determined for all variables. Continuous variables are presented as mean ±SD for normally distributed variables or as median (25%, 75% percentiles) for not normally distributed. For categorical variables, frequency and percentage are reported.

Baseline characteristics (age, weight, BMI, waist circumference, dietary intakes and biomarkers) were compared between countries, by independent t-tests, or Mann-Whitney U tests if appropriate.

For each country separately, ANOVA, or the corresponding non-parametric test Kruskal-Wallis, were used to compare bone parameter measurements between age tertiles and between vitamin D status groups. Pearson’s correlation, or the corresponding non-parametric Spearman rho, were applied to investigate the association between bone parameter measurements and 25(OH)D or PTH concentrations as well as vitamin D and calcium intakes.

Results are presented separately for each country as measurements derived from different methodologies (England: pQCT scan of radius; Brazil: DXA scan of spine and femur).

A p value of <0.05 was considered significant.
5.4 Results

5.4.1 Baseline characteristics

Baseline anthropometric, dietary and biochemical characteristics for this analysis are presented in Table 5.1, by country of residence, due to slightly different sample (excludes menopausal women) than presented previously in Chapter 3. Brazilian women living in England were significantly older \((p<0.001)\), heavier \((p=0.002)\) and had a greater waist circumference \((p<0.001)\) than those living in Brazil.

Overall \((n=114)\), mean habitual vitamin D dietary intake was \(2.44 \pm 1.91 \mu g/day\) and mean calcium intake was \(627 \pm 315 \text{mg/day}\). Mean vitamin D and calcium intakes were significantly higher in England residents compared to Brazil residents \((p<0.001\) and \(p=0.003\), respectively). In total \((n=119)\), 99.2% had intakes below the EAR of 10 \(\mu g/day\) proposed by SACN (74) and by the IOM (71), and 100% had intakes below the RDA of 15 \(\mu g/day\) (71). For calcium, in the overall sample 77.2% had dietary intakes below the RNI of 700 \(\text{mg/day}\) (74), while 91.2% had average intakes below the 800 \(\text{mg/day}\) EAR reference (71).

In participants living in England mean 25(OH)D concentration was significantly lower and PTH concentration was significantly higher than within participants in Brazil. There were no significant differences between Brazilian women living in England and in Brazil for serum calcium concentrations.
Table 5.1 Baseline anthropometric characteristics of adult Brazilian women by country of residence (n=130)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>England (n = 51)</th>
<th>Brazil (n = 79)</th>
<th>p value(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33 (28, 38)</td>
<td>27 (24, 31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.7 (60.7, 73.5)</td>
<td>60.1 (54.1, 71.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>86.3 (77.0, 96.0)</td>
<td>70.4 (66.1, 77.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/cm(^2))</td>
<td>24.6 (22.6, 28.4)</td>
<td>23.3 (20.3, 26.3)</td>
<td>0.009</td>
</tr>
<tr>
<td>Body fat (%)(^5)</td>
<td>30.95 ± 6.00</td>
<td>38.65 ± 8.52</td>
<td>&lt;0.001 (^a)</td>
</tr>
<tr>
<td>Vitamin D (μg/day)</td>
<td>2.59 (1.55, 3.92)</td>
<td>1.57 (0.73, 2.79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/day)</td>
<td>711.76 (537.22, 879.51)</td>
<td>479.97 (347.42, 704.92)</td>
<td>0.003</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/l)</td>
<td>35.2 ± 14.9</td>
<td>75 ± 22.1</td>
<td>&lt;0.001 (^a)</td>
</tr>
<tr>
<td>Plasma PTH (pmol/l)</td>
<td>5.39 ± 2.07</td>
<td>4.49 ± 1.47</td>
<td>0.004 (^a)</td>
</tr>
<tr>
<td>Serum Calcium (mmol/l)(^3)</td>
<td>2.30 ± 0.07</td>
<td>2.28 ± 0.06</td>
<td>0.066 (^a)</td>
</tr>
</tbody>
</table>

\(^1\) Values are median (25\(^{th}\), 75\(^{th}\) percentile) or mean ± SD.
\(^2\) Statistical analysis: Mann-Whitney U unless otherwise stated; \(^a\) Independent t-test
\(^3\) Albumin corrected serum calcium concentrations
\(^5\) Measurements derived from different methodologies (England: bio-impedance; Brazil: DXA scan)

5.4.2 Bone densitometry

5.4.2.1 England cohort

Radial bone parameter measurements, determined by pQCT, for Brazilian women living in England are shown in Table 5.2, stratified by tertile of age. Total volumetric BMD at both distal and diaphyseal sites was significantly higher in women younger than 29 years of age compared to those 30 or over (\(p=0.011\)). There were no significant differences between age groups in any of the other bone parameters. Only one participant in the England cohort (30-36 years old group) had a low t-score for total volumetric BMD at both distal and diaphyseal sites.
Table 5.2 Radial bone densitometry assessed by pQCT of Brazilian women living in England by tertile of age (n= 51)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>Age</th>
<th>Tertile 1 (≤ 29 years)</th>
<th>Tertile 2 (30-36 years)</th>
<th>Tertile 3 (&gt; 37 years)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>51</td>
<td>18</td>
<td>16</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td><strong>Radius distal site (4%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>1.07 ± 0.14</td>
<td>1.05 ± 0.15</td>
<td>1.08 ± 0.13</td>
<td>1.08 ± 0.13</td>
<td>0.821</td>
</tr>
<tr>
<td>Total vBMD (mg/cm$^3$)</td>
<td>353.23± 51.81</td>
<td>380.72 ± 60.82$^a$</td>
<td>329.97 ± 37.07$^a$</td>
<td>346.12 ± 41.47</td>
<td>0.011</td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm$^3$)</td>
<td>199.29 ± 57.78</td>
<td>205.61 ± 73.89</td>
<td>189.48 ± 44.14</td>
<td>201.85 ± 51.53</td>
<td>0.709</td>
</tr>
<tr>
<td><strong>Radius diaphyseal site (66%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>0.96 ± 0.14</td>
<td>0.93 ± 0.15</td>
<td>0.97 ± 0.12</td>
<td>0.97 ± 0.14</td>
<td>0.557</td>
</tr>
<tr>
<td>Total vBMD (mg/cm$^3$)</td>
<td>714.38 ±86.33</td>
<td>753.33 ± 68.57</td>
<td>693.10 ± 90.80</td>
<td>693.17 ± 89.02</td>
<td>0.056</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm$^3$)</td>
<td>1127.23 ± 36.44</td>
<td>1140.09 ± 27.03</td>
<td>1124.26 ± 37.61</td>
<td>1116.43 ± 41.59</td>
<td>0.147</td>
</tr>
<tr>
<td><strong>Calculated T-score n(%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total vBMD (4%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (&gt; -1.0)</td>
<td>50 (98%)</td>
<td>18 (100%)</td>
<td>16 (94.8%)</td>
<td>17 (100%)</td>
<td></td>
</tr>
<tr>
<td>Low (-1.0 – -2.5)</td>
<td>1 (2%)</td>
<td>0</td>
<td>1 (5.2%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis &lt; 2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Trabecular vBMD (4%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (&gt; -1.0)</td>
<td>51 (100%)</td>
<td>18 (100%)</td>
<td>16 (100%)</td>
<td>17 (100%)</td>
<td></td>
</tr>
<tr>
<td>Low (-1.0 – -2.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis &lt; 2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Total vBMD (6%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (&gt; -1.0)</td>
<td>50 (98%)</td>
<td>18 (100%)</td>
<td>16 (94.8%)</td>
<td>17 (100%)</td>
<td></td>
</tr>
<tr>
<td>Low (-1.0 – -2.5)</td>
<td>1 (2%)</td>
<td>0</td>
<td>1 (5.2%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis &lt; 2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Cortical vBMD (66%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (&gt; -1.0)</td>
<td>56 (100%)</td>
<td>18 (100%)</td>
<td>16 (100%)</td>
<td>17 (100%)</td>
<td></td>
</tr>
<tr>
<td>Low (-1.0 – -2.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis &lt; 2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

1 Values: mean ± SD or n(%); 2 Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same column with same superscript letters are significantly different (\(^a\) p <0.001).
5.4.2.2 Brazil cohort

Lumbar spine (L1-L4) and femur measurements, determined by DXA, for Brazilian women living in Brazil are shown in Table 5.3, stratified by tertile of age. There were no significant differences between age groups in any of the bone parameters. Overall, 25% and 18% of participants had a low t-score for lumbar spine and femur, respectively, with the majority of those being under 30 years old. Those with a low t-score had also predominantly a healthier BMI, with statistical significance for lumbar spine (90.5% BMI < 25 kg/m², Pearson Chi-square= 0.038), but not for femur (72.7% BMI < 25 kg/m² Pearson Chi-square=0.912).

Table 5.3 Lumbar spine and femur bone densitometry assessed by DXA of Brazilian women living in Brazil by tertile of age (n=79)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>Age</th>
<th>Lumbar Spine</th>
<th>Femur</th>
<th>Total BMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>Tertile 1 &lt;=25</td>
<td>Tertile 2 26 - 30</td>
<td>Tertile 3 &gt;31</td>
</tr>
<tr>
<td>Number (n)</td>
<td>79</td>
<td>33</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>BMD</td>
<td>1.13 ± 0.11</td>
<td>1.12 ± 0.12</td>
<td>1.13 ± 0.11</td>
<td>1.15 ± 0.11</td>
</tr>
<tr>
<td>Number (n)</td>
<td>64</td>
<td>27</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>BMD</td>
<td>0.98 ± 0.10</td>
<td>0.97 ± 0.10</td>
<td>0.97 ± 0.12</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>Number (n)</td>
<td>79</td>
<td>33</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>BMC</td>
<td>2304.87 ± 401.76</td>
<td>2304.87 ± 401.76</td>
<td>2459.92 ± 377.29</td>
<td>2460.95 ± 338.42</td>
</tr>
<tr>
<td>T-score n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar Spine BMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (&gt; -1.0)</td>
<td>58 (73.4%)</td>
<td>23 (69.7%)</td>
<td>18 (72%)</td>
<td>13 (81%)</td>
</tr>
<tr>
<td>Low (-1.0 – -2.5)</td>
<td>20 (26.6%)</td>
<td>9 (27.3)</td>
<td>7 (28%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>Osteoporosis &lt; 2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Femur BMD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (&gt; -1.0)</td>
<td>52 (81.2%)</td>
<td>22 (81.5%)</td>
<td>15 (75%)</td>
<td>15 (88.2%)</td>
</tr>
<tr>
<td>Low (-1.0 – -2.5)</td>
<td>12 (18.8%)</td>
<td>5 (18.5%)</td>
<td>5 (25%)</td>
<td>2 (11.8%)</td>
</tr>
<tr>
<td>Osteoporosis &lt; 2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Values: mean ± SD or n(%); 2 Statistical analysis: one-way ANOVA with post-hoc Tukey’s test.
5.4.3 Correlations between bone parameters and age and adiposity

5.4.3. England cohort

BMC at radius distal site (4%) BMC at the distal site (4%) was positively correlated with weight and BMI (p=0.021 and p=0.024 respectively). Total vBMD at the diaphyseal site (66%) and cortical vBMD were negatively correlated with weight (p=0.016 and p=0.025 respectively) and BMI (p=0.018 and p=0.027 respectively) (Table 4). When weight and BMI were entered into a regression model as predictors of BMC at the distal site, the model did not achieve statistical significance to explain the variation (p=0.068). When weight and BMI were entered into a regression model as predictors of total vBMD at the diaphyseal site, 33.7% (p=0.055) of the total variation was explained by the model but none of the predictors had a significantly unique contribution. When weight and BMI were entered into a regression model as predictors of cortical vBMD, the model did not achieve statistical significance to explain the variation (p=0.08).

Table 5.4 Correlations between age and adiposity and radial bone densitometry assessed by pQCT of Brazilian women living in England by tertile of age (n= 51)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Body fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td>Radius distal site (4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>0.186</td>
<td>0.191</td>
<td>0.323</td>
<td><strong>0.021</strong></td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>-0.143</td>
<td>0.316</td>
<td>-0.005</td>
<td>0.970</td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm³)</td>
<td>-0.007</td>
<td>0.962</td>
<td>-0.118</td>
<td>0.410</td>
</tr>
<tr>
<td>Radius diaphyseal site (66%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>0.216</td>
<td>0.128</td>
<td>0.256</td>
<td>0.070</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>-0.213</td>
<td>0.134</td>
<td>-0.335</td>
<td><strong>0.016</strong></td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>-0.223</td>
<td>0.116</td>
<td>-0.313</td>
<td><strong>0.025</strong></td>
</tr>
</tbody>
</table>
5.4.3.2 Brazil cohort

Age was positively correlated with BMD at the femur and with BMC (p=0.029 and p=0.017, respectively), but not BMD at the lumbar spine (p=0.292). Weight, BMI and body were correlated with BMD at the lumbar spine and femur and with BMC (all p<0.01). (Table 5). When age along with either weight, BMI or body fat were entered into a regression model as predictors of BMD at the femur, 64.3% (p<0.001) of the total variation was explained by the model, and only BMI had a significantly unique contribution (p=0.027). When age along with weight, BMI or body fat were entered into a regression model as predictors of BMC, 67% (p<0.001) of the total variation was explained by the model, and only weight and BMI had a significantly unique contributions to the model (p<0.001 and p=0.004, respectively). When weight, BMI and body fat were entered into a regression model as predictors of BMD at the lumbar spine, 34.2% (p=0.025) of the total variation was explained by the model but none of the predictors had a significantly unique contribution.

Table 5.5 Correlations between age and adiposity and lumbar spine and femur bone densitometry assessed by DXA of Brazilian women living in Brazil by tertile of age (n= 79)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Body fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td>Lumbar Spine BMD (n=79)</td>
<td>0.120</td>
<td>0.292</td>
<td>0.337</td>
<td>0.002</td>
</tr>
<tr>
<td>Femur BMD (n=64)</td>
<td>0.273</td>
<td><strong>0.029</strong></td>
<td>0.583</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Total BMC (n=79)</td>
<td>0.267</td>
<td><strong>0.017</strong></td>
<td>0.606</td>
<td><strong>&lt;0.001</strong></td>
</tr>
</tbody>
</table>

5.4.4 Correlations between bone parameters and habitual dietary vitamin D and calcium intakes

5.4.4.1 England cohort

There were no significant correlations between radial bone parameter measurements, determined by pQCT, and habitual dietary vitamin D and calcium intakes for Brazilian women living in England (Table 5.6).
Table 5.6 Correlations between habitual dietary vitamin D and calcium intakes and radial bone densitometry assessed by pQCT of Brazilian women living in England by tertile of age (n= 51)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>Vitamin D intakes</th>
<th>Calcium intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td><strong>Radius distal site (4%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>0.172</td>
<td>0.254</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>0.195</td>
<td>0.194</td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm³)</td>
<td>0.189</td>
<td>0.209</td>
</tr>
</tbody>
</table>

**Radius diaphyseal site (66%)**

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>Vitamin D intakes</th>
<th>Calcium intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>0.255</td>
<td>0.088</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>0.117</td>
<td>0.439</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>0.097</td>
<td>0.523</td>
</tr>
</tbody>
</table>

5.4.4.2 Brazil cohort

There were no significant correlations between lumbar spine (L1-L4) and fêmur bone parameter measurements, determined by DXA, and habitual dietary vitamin D and calcium intakes for Brazilian women living in Brazil (Table 5.7).

Table 5.7 Associations between habitual dietary vitamin D and calcium intakes and lumbar spine and femur bone densitometry assessed by DXA of Brazilian women living in Brazil by tertile of age (n= 79)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>Vitamin D intakes</th>
<th>Calcium intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td><strong>Lumbar Spine (L1-L4) BMD (n=68)</strong></td>
<td>0.137</td>
<td>0.264</td>
</tr>
<tr>
<td><strong>fêmur BMD (n=55)</strong></td>
<td>0.216</td>
<td>0.113</td>
</tr>
<tr>
<td><strong>Total BMC (n=68)</strong></td>
<td>0.013</td>
<td>0.918</td>
</tr>
</tbody>
</table>
5.4.5 Associations between bone parameters and 25(OH)D concentrations

Correlations between bone parameter measurements and 25(OH)D concentrations are presented separately for each country as measurements derived from different methodologies (England: pQCT scan of radius; Brazil: DXA scan of spine and fēmur).

5.4.5.1 England cohort

There was a trend for a weak positive relationship between total volumetric BMD at the diaphyseal site and 25(OH)D concentrations, but significance was lost after controlling for age and BMI ($p=0.171$). There were no significant correlations between any other bone parameter measurements (Table 5.8).

Table 5.8 Associations between 25(OH)D concentrations and radial bone densitometry assessed by pQCT of Brazilian women living in England (n= 51)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>25(OH)D</th>
<th>r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radius distal site (4%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td></td>
<td>0.089</td>
<td>0.537</td>
</tr>
<tr>
<td>Total vBMD (mg/cm$^3$)</td>
<td></td>
<td>-0.084</td>
<td>0.562</td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm$^3$)</td>
<td></td>
<td>0.017</td>
<td>0.908</td>
</tr>
<tr>
<td><strong>Radius diaphyseal site (66%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td></td>
<td>-0.180</td>
<td>0.214</td>
</tr>
<tr>
<td>Total vBMD (mg/cm$^3$)</td>
<td></td>
<td>0.278</td>
<td><strong>0.051</strong></td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm$^3$)</td>
<td></td>
<td>0.173</td>
<td>0.229</td>
</tr>
</tbody>
</table>
5.4.5.2 Brazil cohort

There were no significant correlations between lumbar spine (L1-L4) and fêmur bone parameter measurements, determined by DXA, and 25(OH)D concentrations for Brazilian women living in Brazil (Table 5.9).

Table 5.9 Associations between 25(OH)D concentrations and lumbar spine and femur bone densitometry assessed by DXA of Brazilian women living in Brazil (n= 79)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
</tr>
<tr>
<td>Lumbar Spine (L1-L4) BMD (n=79)</td>
<td>0.060</td>
</tr>
<tr>
<td>femur BMD (n=64)</td>
<td>0.059</td>
</tr>
<tr>
<td>Total BMC (n=79)</td>
<td>0.101</td>
</tr>
</tbody>
</table>

5.4.6 Associations between bone parameters and vitamin D status

5.4.6.1 England cohort

When stratified by baseline vitamin D status as previously defined in this thesis (Table 2.5, section 2.4.7, Chapter 2), women with vitamin D deficiency (<25 nmol/l) had significantly lower total (p=0.0019) and cortical (p=0.039) vBMD at the diaphyseal site than women classed as insufficient (25 – 49.9 nmol/l), and significantly lower total vBMD at the diaphyseal site than women with levels above 50 nmol/l (0.044) (Table 5.10). After controlling for age and BMI, the significant differences remained only for total vBMD at the diaphyseal site but not for cortical vBMD (ANCOVA p =0.047 and p=0.170, respectively).
Table 5.10 Radial bone densitometry assessed by pQCT of Brazilian women living in England by vitamin D status (n= 51)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>25(OH)D in nmol/l</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>Number (n)</td>
<td>51</td>
<td>14</td>
</tr>
<tr>
<td>Radius distal site (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>1.07 ± 0.14</td>
<td>1.06 ± 0.12</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>352.23 ± 51.81</td>
<td>355.81 ± 40.47</td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm³)</td>
<td>199.29 ± 57.78</td>
<td>189.56 ± 25.61</td>
</tr>
<tr>
<td>Radius diaphyseal site (66%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>0.96 ± 0.14</td>
<td>0.97 ± 0.13</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>714.39 ± 86.33</td>
<td>658.54 ± 104.23</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>1127.23 ± 36.44</td>
<td>1107.74 ± 44.43</td>
</tr>
</tbody>
</table>

¹Values: mean ± SD
²Statistical analysis: one-way ANOVA with post-hoc Tukey’s test. Values in same row with same superscript letters are significantly different (*p = 0.019, †p = 0.044, ‡p = 0.039).

5.4.6.2 Brazil cohort

There were no significant differences in any bone parameter measurements by vitamin D status, for Brazilian women living in Brazil (Table 5.11).

Table 5.11 Lumbar spine and fémur bone densitometry assessed by DXA of Brazilian women living in Brazil by vitamin D status (n= 79)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>25(OH)D in nmol/l</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>25 – 49.99</td>
</tr>
<tr>
<td>Lumbar Spine (L1-L4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (n)</td>
<td>79</td>
<td>9</td>
</tr>
<tr>
<td>BMD</td>
<td>1.13</td>
<td>1.11</td>
</tr>
<tr>
<td>±0.12</td>
<td>±0.11</td>
<td>±0.12</td>
</tr>
<tr>
<td>Femur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (n)</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>BMD</td>
<td>0.98</td>
<td>0.94</td>
</tr>
<tr>
<td>± 0.10</td>
<td>±0.10</td>
<td>±0.10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (n)</td>
<td>79</td>
<td>9</td>
</tr>
<tr>
<td>BMC</td>
<td>23.95 ± 305.66</td>
<td>2391.46</td>
</tr>
<tr>
<td>381.24 ± 448.47</td>
<td>331.93</td>
<td></td>
</tr>
</tbody>
</table>

¹Values: mean ± SD
²Statistical analysis: one-way ANOVA with post-hoc Tukey’s test.
5.4.7 Associations between bone parameters and PTH concentrations

As previously reported, PTH concentrations were negatively correlated with 25(OH)D concentrations (r = -0.285, p = 0.001). A non-linear regression cubic model was found to have the best fit (n = 130, R² = 0.113, p = 0.001), with a PTH reaching a minimum plateau at 25(OH)D concentrations of over 75 nmol/l (Section 3.4.9, Chapter 3). Overall (n=134), 10.4% of participants presented with secondary hyperparathyroidism (PTH concentrations > 6.9 pmol/l). The prevalence of secondary hyperparathyroidism according to vitamin D status was 28.6% (n=14) in vitamin D deficient participants (<25 nmol/l) and 12.2% (n=41) within vitamin D insufficient participants (25 – 49.9 nmol/l). Only 3 (5%) participants with adequate levels (50 – 74.9 nmol/l) and 2 (2.6%) participants with optimal levels (> 75 nmol/l) presented with secondary hyperparathyroidism (p=0.086 for comparison of prevalence across status groups). (Figure 5.1)

Figure 5.1 Prevalence of secondary hyperparathyroidism (PTH > 6.9 pmol/l) at baseline according to vitamin D status in Brazilian women recruited to a vitamin D supplementation study (n=134)
5.4.7.1 England cohort

Correlations between radial bone parameter measurements, determined by pQCT, and PTH concentrations for Brazilian women living in England are shown in Table 5.12. There was a significant positive association between PTH concentrations and BMC at both distal and diaphyseal sites (p=0.0012 and p =0.001, respectively). Total vBMD at the diaphyseal site was significantly negatively correlated with PTH concentrations (p=0.026). However, after controlling for age and BMI significance remained only for BMC at diaphyseal site (p=0.039) but not for BMC at distal site or total vBMD at the diaphyseal site (p=0.064 and p=0.078, respectively).

Table 5.12 Associations between PTH concentrations and radial bone densitometry assessed by pQCT of Brazilian women living in England (n= 51)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
</tr>
<tr>
<td>Radius distal site (4%)</td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>0.348</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>0.108</td>
</tr>
<tr>
<td>Trabecular vBMD (mg/cm³)</td>
<td>0.085</td>
</tr>
<tr>
<td>Radius diaphyseal site (66%)</td>
<td></td>
</tr>
<tr>
<td>BMC (g/cm)</td>
<td>0.435</td>
</tr>
<tr>
<td>Total vBMD (mg/cm³)</td>
<td>-0.312</td>
</tr>
<tr>
<td>Cortical vBMD (mg/cm³)</td>
<td>-0.184</td>
</tr>
</tbody>
</table>

5.4.7.2 Brazil cohort

There were no significant correlations between lumbar spine (L1-L4) and femur bone parameter measurements, determined by DXA, and PTH concentrations for Brazilian women living in Brazil (Table 5.13).
Table 5.13 Correlations between PTH concentrations and lumbar spine and femur bone densitometry assessed by DXA of Brazilian women living in Brazil (n= 78)

<table>
<thead>
<tr>
<th>Bone Parameter</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
</tr>
<tr>
<td>Lumbar Spine (L1-L4) BMD (n=78)</td>
<td>0.004</td>
</tr>
<tr>
<td>Femur BMD (n=63)</td>
<td>0.215</td>
</tr>
<tr>
<td>Total BMC (n=78)</td>
<td>0.114</td>
</tr>
</tbody>
</table>
5.5 Discussion

Vitamin D is essential for musculoskeletal health, and there is a consensus that serum concentrations should be at least 25 nmol/l to prevent detrimental effects to bone. The present cross-sectional analysis of healthy Brazilian adult women showed good bone health in women living in England, but 18% and 25% of women in Brazil presented with a low t-score for lumbar spine and femur, respectively. As a result of Chi-square test analysis, higher proportion of these women with a BMI < 25 kg/m² was observed in the lower BMD t-score group compared to normal t-score, however the difference was statistically significant only for lumbar spine. Furthermore, association with bone parameters were significantly associated with adiposity in participants in the England cohort and with adiposity and age in the Brazil cohort. This study also found that amongst women living in England those with vitamin D deficiency (<25 nmol/l) had significantly lower total vBMD at the diaphyseal radius than those with greater concentrations, independent of age and BMI. There were no associations between 25(OH)D status and any of the measures from DXA scan in participants from the Brazil cohort although the range of Vitamin D status was smaller and the generally better status of deficiency much lower which may have limited the effects on bone.

The low t-score associated with a BMI < 25 kg/m² and associations between bone parameters and adiposity suggest that the observed poorer bone health in this sample is likely to be due to lower weight. These findings are in agreement with several of studies that have demonstrated positive association of BMI with BMD that is likely explain by a greater load on weight-bearing bones (162–165). In a cross-sectional study, a total of 412 Brazilian postmenopausal women, aged 40-75 years, with BMD assessed by DXA at the lumbar spine, higher BMI reduced risk for osteoporosis (166). A study with 393 post-menopausal Brazilian women reported a lower prevalence of osteopenia and osteoporosis amongst obese women compared to those with eutrophic BMI (DXA-derived BMD assessment) (167). Another study in São Paulo, with 413 Brazilian women (52.5% < 59 years and 47.5% > 60 years) BMI was shown to be a positive predictor for DXA-derived BMD at the femoral neck (168).
A considerable amount of human evidence supports the well-recognized and intuitive contribution of calcium intake for bone homeostasis maintenance. Human supplementation cohorts have showed that higher calcium intake in the long-term could have relevant benefits to bone health and reduce the risk of osteoporosis by slowing the rate of bone loss associated with aging (169), and effects might be strongest in postmenopausal women, rather than peri-menopausal (early stages) (170). In the United Kingdom, the latest national survey reported mean dietary calcium intakes of 897 and 746 mg/day for men and women aged 19 - 64 years, respectively (NDNS years 7-8, 2015–2016)(171). In Brazil, the latest national survey available to date reported that mean dietary calcium intakes were 546.4 and 476.4 mg/day for men and women aged 20 - 59 years, respectively (POF, 2008-2009)(109).

To date, there are still few longitudinal studies that have investigated the association of long-term lower calcium intake bone health outcomes later in life, particularly from a younger age in adulthood i.e. < 30-35 years. A longitudinal study 5022 women (born between 1914 and 1948 and followed up for 19 years) and with modest dietary calcium intake reported that only the lowest quintile of calcium intake was associated with increased risk of fracture or osteoporosis (172). It has been suggested that the association between calcium and BMD might not be consistently linear, and a sufficient vitamin D level are likely to compensate for the negative effects of low calcium intake on bone (173–175).

In this cohort, 72.8% of participants had dietary calcium intakes below the RNI of 700 mg/day, while only 5.2% met the 1000 mg/day RDA reference, whilst 100% of participants had dietary vitamin D intakes below the 15 μg/day RDA recommended by the IOM (71) and 99.2% had intakes below the RNI of 10 μg/day proposed by SACN (74). Despite this there were no significant correlations between bone parameter measurements and habitual dietary vitamin D and calcium intakes, either for England or Brazil dwelling participants perhaps again reflecting the narrow range of intakes and therefore lack of discriminatory power in this sample.

Elevated concentrations of serum PTH are associated with several adverse outcomes, particularly musculoskeletal (35,89,176). Secondary hyperparathyroidism may lead to bone loss due to increased bone turnover rates (35,177). Several studies have shown
25(OH)D to be inversely correlated with PTH (8,35,135,178). The present study confirms this with 25(OH)D concentration being inversely correlated with PTH concentration. Additionally, 10.4% of participants had secondary hyperparathyroidism, with a higher prevalence amongst those with deficient and insufficient vitamin D status.

Moreover, several studies have also shown an inverse correlation between serum PTH level and BMD (135,177,179). In this study, a negative correlation between PTH concentrations and total BMD at the diaphyseal radius site was seen, although this was not significant after controlling for age and BMI. There were no associations with PTH and any of the measures from the DXA scan in participants from the Brazil cohort, which may be due to long-term detrimental consequences of higher PTH levels still not evident in the relative young women in these study.

The associations between 25(OH)D, PTH and bone mineral density (BMD) are still much debated. Evidence is more robust in particular subgroups such as in those with low vitamin D levels (180), in osteoporotic subjects (50,51,152), post-menopausal women (50,135,181) or in the elderly (35,157,182). The findings presented here are in accordance with previous reports of studies that included healthy younger adults as well as those that encompass the whole spectrum of vitamin D status (i.e., deficient, sufficient and adequate), demonstrating the absence of any association between 25(OH)D or PTH and BMD (156,183,184). The reasons for no effects on bone parameters might be due to the lack of power within the deficient subgroup and the relatively young participants, who might not be currently affected by potential long-term detrimental outcomes from low 25(OH)D and high PTH concentrations.

5.5.1 Strengths and limitations

Strengths of this study includes the use of pQCT and DXA scan, both considered gold standard measurements for bone density, controlling for possible cofounders such as age and BMI. Although published reference data were used to calculate t-scores based on the recommendations for the Brazilian population (white American adult women), the DXA scan software reports using a combination of databases to calculated the t-score, while for the pQCT data a specific published reference data was used. Ideally,
the method would have been used for both groups, and pQCT would have been the preferred option, were it available at the Clinical Investigation Unit in Brazil as well, as it is the gold-standard method and has been shown to provide a greater diagnostic sensitivity than DXA (185). This analysis would benefit from further measures of biochemical markers of bone turnover to further investigate the relationship of 25(OH)D, PTH and bone health in these adult women.

Conclusions

In conclusion, adiposity was associated with bone parameters in healthy adult Brazilian women, assessed by pQCT in England-dwelling participants and DXA-derived in Brazil-dwelling participants. This analysis also showed that secondary hyperparathyroidism was more common amongst those with deficient and insufficient vitamin D status.
6 Final Discussion and Conclusions

6.1 Summary of thesis results and original contribution

It is widely recognized that vitamin D inadequacy is a global public health issue and that whilst populations at higher latitudes are at greater risk of deficiency particularly during winter, the concern is now extended to tropical locations too. The issue appears in low latitude countries as a paradoxical situation with sunlight underexposure leading to vitamin D inadequacy while sunlight overexposure may lead to skin damage, aging and cancer. Targeting intervention strategies specific to each population, country and ambient setting is important if the balance of risk and benefit, of both sunlight exposure and vitamin D supplementation, is to be optimised. Therefore, studies specifically designed to investigate the effect of, and requirements for, vitamin D supplements and sunlight exposure in sunny countries are urgently required to contribute to an improved definition of vitamin D deficiency and country-specific recommendations.

This is the first study to investigate the effect of vitamin D supplementation and individual sunlight exposure in healthy Brazilian adult women living in two opposing latitudes and the first to estimate vitamin D intake and sunlight exposure required to meet concentrations of 75 nmol/l in this population group in Brazil. The findings in this thesis confirm that both sunlight exposure and vitamin D supplementation can contribute greatly to achieving and/or maintaining optimal concentrations of serum 25(OH)D. The present study demonstrates a strong positive correlation between individual UV radiation level and vitamin D serum concentrations. Furthermore, a relatively low daily dose of vitamin D via a supplement has an important positive impact on winter vitamin D status in adult women, regardless of latitude. Supplemental 15 μg of vitamin D daily, for three months, significantly increased serum 25(OH)D concentrations and prevented seasonal increase in PTH concentrations, in a high
latitude as well as in a low latitude country. This relatively low dose was effective and therefore the intake of significantly larger doses, as has been recently proposed, to increase 25(OH)D is not supported by this data, regardless of latitude.

More specifically, this study showed an optimal vitamin D status of 70-80 nmol/l for Brazilian adult women, based on the suppression of PTH. An individual UV radiation level of 0.28 SED was estimated as required to meet a serum 25(OH)D concentration of 50 nmol/l and 1.5 SED for a serum 25(OH)D concentration of 75 nmol/l, when only individual UV radiation was included in the model. For every 1 μg (40 IU) of vitamin D supplemented it would be expected an increase in 25(OH)D concentrations of 1.002 nmol/l if living in a high latitude and 0.791 nmol/l if living in a low latitude for adult Brazilian women, when only vitamin D intake was included to the model.

Nevertheless, this study has also evidenced that the recommended dose for vitamin D supplementation should not only be country-specific but, more importantly, take into consideration mainly the initial serum 25(OH)D concentrations of the individual. Moreover, recommendations must also take into account the availability of each vitamin D source (dietary intake, either from food or supplements, and endogenous production from sunlight exposure), individual characteristics and lifestyle aspects. The development of country specific risk scores for vitamin D deficiency, which would take into consideration all the aspects here discussed of vitamin D synthesis, metabolism and intake, might be a strategy to target those that would need and benefit more from vitamin D supplementation. Additionally, even if sunlight availability is not limited, it is important to identify situations where increasing sunlight exposure will not result in vitamin D status improvement, i.e. individual variation in cutaneous production, and vitamin D supplementation must be considered as an alternative options.

6.2 Study impact and future directions

Most recommendations on vitamin D intake and status assume no significant cutaneous synthesis of vitamin D through sun exposure. This is true in high latitudes during winter months, but in tropical countries individual UV radiation is likely to be high, and at a
sufficient minimum to produce vitamin D adequately throughout the year. Therefore, in low latitude countries the contribution of UV exposure must be considered when estimating optimal vitamin D intakes for populations.

The data here presented provides robust and specific information that can contribute greatly to establishing specific recommendations for Brazil by the authoritative bodies responsible for formulating nutrition policy and public health guidelines. Specifically, the data generated in the present study: 1) confirms individual sunlight exposure as an important source of vitamin D in Brazil, even during winter; 2) indicates an optimal vitamin D status of 70-80 nmol/l for adult Brazilian women; 3) shows that supplementation is most helpful in persons with lower baseline concentrations; 4) suggests that a vitamin D daily intake of approximately a third of the current recommendation of 15 μg, based on US guidelines, is required by healthy adult Brazilian women at latitude of ≈16 °S to maintain 25(OH)D concentrations above 75 nmol/l. In contrast, vitamin D daily intakes of 15 μg seem to be adequate to maintain 25(OH)D concentrations above 50 nmol/l in Brazilian adult women at latitude of ≈51 °N, although over twice as much (37 μg) would be required for concentrations above 75 nmol/l.

Furthermore, while food fortification can be useful and effective in increasing vitamin D status in populations, this may not the be an appropriate strategy in a low latitude country. This is an important aspect of the discussion on the need for country-specific recommendations supported by the findings presented here. In low latitude countries, it is unlikely that the need for additional vitamin D is universal, as it may be for other nutrients that are added to foods (such as sodium in Brazil). Moreover, high concentrations of 25(OH)D are already observed in the Brazilian population (over 100 nmol/l) and food fortification strategies could lead to overdosing and potentially toxic levels. Vitamin D supplementation might be a better targeted strategy in tackling particular cases of deficiency.

Further vitamin D RCTs are now required in Brazil to help determine a general consensus on vitamin D recommendations for the wider Brazilian population, and to confirm whether the predicted values in this study can be generalised. Further investigation on the seasonal cycling of vitamin D concentrations in low latitudes is
also required to understand the interactions and necessity of vitamin D intake in this population throughout the year.

Detailed investigations of environmental, biological and behavioural factors affecting vitamin D synthesis would provide valuable data for the development of vitamin D deficiency risk assessment tools. From a public health perspective this is of great relevance in a country such Brazil, which has approximately 200 million people and of which the majority relies on the public national health system. Such tools could potentially optimize detection of potential inadequacy and initiate treatment or prevention, without having to rely on biochemical analysis and waiting time for assessments, also reducing significantly the costs of vitamin D testing.

6.3 Critical evaluation of the study

The strengths and limitations of each analysis have been discussed in the respective chapters. A critical evaluation of the overall study design and protocol will be considered in this section.

The RCT was specifically designed to investigate the effectiveness of vitamin D supplementation in improving vitamin D status by increasing mean 25(OH)D serum concentrations in the range of 20 -25 nmol/l, over wintertime in opposite latitudes. The power calculation, based on previously published research identified that a sample size of 64 women for each country plus a 16 women to account for a 20% dropout rate, to demonstrate a response to vitamin D supplementation in the range of 20 -25 nmol/l at $\alpha = 0.05$. In total 56 women in the England trial and 80 women in the Brazil trial were enrolled in the study.

Recruitment for the England trial was more difficult, particularly due to the need for participants to take a train to attend the visits at the University of Surrey, as only 4 participants were actually Guildford residents. This seems to have been the main factor as well for participants booked to their first visit not showing up at all. The use of social media to advertise the study proved to be the most efficient method of recruitment, in
both countries, and seems to have reached a broader public than it would have been with only posters on local establishments.

The study protocol for the RCTs described in Chapter 2 and available in Appendix II, was followed rigorously throughout the study. The low dropout rate and satisfactory compliance was likely due to the regular communication maintained with participants throughout the duration of the study via weekly text messages, in both countries. Participants were instructed to return any missed supplements and informed that it was not a problem to miss any supplements as long as they were honest with the research team to ensure accurate measures of compliance.

Blood samples were processed following the same protocol in both countries and stored frozen until being sent to analysis at Imperial College, London. Samples collected in Brazil were transported to the UK on dry ice by air via a specialized company (World Courier Group, Inc.) that assisted with all the paperwork and sample preparation for transport. The transportation box contained 2 digital thermometers that registered the temperature periodically throughout the whole journey.

There were some minor issues with the lifestyle questionnaire, with some participants needing clarification for a few questions, as already mentioned in previous chapters. This suggests that the questionnaire could have been better tailored to this population. Also, some questions could have included more details, as already discussed in previous chapters, to better address the particularities of the two countries and the population studied.

6.4 Study hypothesis critical review

**Hypothesis 1**: Dietary intakes of vitamin D are too low and sunlight exposure is insufficient for maintaining optimal vitamin D status throughout wintertime in Brazilian women, regardless of latitude of residence. A high prevalence of women below the vitamin D sufficiency threshold was observed amongst those living in England, but vitamin D status in women living in Brazil was generally adequate.
Vitamin D intake was extremely low in both countries and sunlight exposure was strongly associated with vitamin D serum concentrations, and below 1 SED in all women living in England and in half of the women living in Brazil.

**Hypothesis 2:** Vitamin D supplementation is effective in raising and maintaining adequate 25(OH)D concentrations throughout winter, regardless of latitude. Vitamin D supplementation was significantly effective in raising and maintaining mean serum vitamin D above the sufficiency threshold, compared to no significant changes in the placebo, in both latitudes over wintertime.

**Hypothesis 3:** The response of Brazilian women to vitamin D supplementation is dependent on baseline 25OHD levels. Baseline concentration was the major contributor to the variation in the response to total vitamin D intake.

**Hypothesis 4:** Circulating 25(OH)D and PTH concentrations are associated with bone health parameters. Circulating 25(OH)D was significantly negatively associated with PTH concentrations, but no associations between these two biomarkers and bone parameters were observed.
Concluding remarks

Effective and meaningful evidence-based vitamin D recommendations are of great importance to prevent vitamin D deficiency and long-term detrimental effects to bone health especially. Moreover, country and context specific recommendations are crucial to establish more precise requirements for vitamin D intake, sunlight exposure and optimal status.

The main focus of the study was whether vitamin D supplementation in a high latitude country differed from a low latitude country with respect to response depending on individual sunlight exposure and whether there was a benefit compared to the placebo group regardless of latitude. This thesis provides robust evidence on the differences in response and the beneficial effect of vitamin D supplement in high latitude compared to low latitude was demonstrated as proposed. Furthermore, it provides evidence for an optimal vitamin D status for bone health of 75 nmol/l, a greater response to supplementation in those with lower concentrations, a required 1.5 SED for a serum 25(OH)D concentration of 75 nmol/l and a required vitamin D daily intake of 4.5 μg at latitude of ≈16 °S to maintain 25(OH)D concentrations above 75 nmol/l and of 15 and 37 μg to maintain 25(OH)D concentrations above 50 and 75 nmol/l, respectively at latitude of ≈51 °N, in Brazilian adult women.

In conclusion, this study provides strong evidence of the need for country specific recommendations for optimal vitamin D status and confirms that the response to vitamin D supplementation, particularly in a sunny country, depends largely on initial vitamin D serum concentrations.
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Table I.1 Serum 25OHD, plasma PTH and serum calcium concentrations at baseline and post-intervention in England and Brazil trials, Per Protocol Analysis (n=95)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>England</th>
<th>Brazil</th>
<th>(p^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo 15 µg/day</td>
<td>Placebo 15 µg/day</td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>32.40 ± 14.68</td>
<td>37.56 ± 16.51</td>
<td>0.313</td>
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<tr>
<td>Post-intervention</td>
<td>39.22 ± 16.62</td>
<td>55.29 ± 12.40</td>
<td>0.001</td>
</tr>
<tr>
<td>Total Change</td>
<td>6.82 ± 10.66</td>
<td>17.73 ± 17.01</td>
<td>0.026</td>
</tr>
<tr>
<td>(p^5)</td>
<td>0.018</td>
<td>&lt;0.001</td>
<td>0.619</td>
</tr>
</tbody>
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|                         |                             |                             |           |
| Plasma PTH (pmol/l)     |                              |                             |           |
| Baseline                | 5.60 ± 1.40                  | 5.42 ± 2.71                 | 0.796     |
| Post-intervention       | 6.43 ± 17.1                  | 5.91 ± 2.30                 | 0.440     |
| Total Change            | 0.82 ± 1.90                  | 0.23 ± 2.43                 | 0.412     |
| \(p^5\)                | 0.092                        | 0.339                       | 0.003     |

| Serum Calcium (mmol/l)  |                              |                             |           |
| Baseline                | 2.30 ± 0.07                  | 2.29 ± 0.08                 | 0.483     |
| Post-intervention       | 2.30 ± 0.06                  | 2.28 ± 0.06                 | 0.249     |
| Total Change            | -0.01 ± 0.05                 | -0.01 ± 0.06                | 0.815     |
| \(p^5\)                | 0.705                        | 0.448                       | 0.311     |

\(^1\) Values mean ± SD; analysis of intention to treat
\(^2\) Albumin corrected serum calcium concentrations
\(^3\) Statistical analysis:
\(^4\) Between intervention group within each country: Independent t-test;
\(^5\) Between baseline and week 12: Paired t-test
Figure I.1 Individual response to intervention according to baseline serum 25(OH)D concentrations by intervention group and country A) England Placebo; B) England 15 µg/d; C) Brazil Placebo; D) Brazil 15 µg/d.
Appendix II

D-SOL Study Documentation

- D-SOL Study Protocol
- University of Surrey Ethics Committee approval letter
- University of Goiás Ethics Committee approval letter
- Brazilian National Ethics Committee (CONEP) approval letter
- Consent form
- Recruitment poster
- Invitation letter
- Participant Information Sheet
- Screening Questionnaire
- Lifestyle questionnaire
Detailed Protocol

Protocol Title:
A systems biology approach to the interaction between vitamin D supplementation and sunlight exposure in Brazilian women living in opposite latitudes (The D-SOL Study).

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1.0 Background & Rationale

Vitamin D is derived from two sources: the skin (endogenous) and the diet (exogenous). It is generally believed that the major source of vitamin D is the exposure of the skin to UVB-rays contained in sunlight. However, the full characterization of the contribution of diet and sunlight to optimal vitamin D status remains unknown. Furthermore, the response of individuals to vitamin D supplementation is known to be variable in all population groups since several personal and environmental characteristics may affect the conversion of pre-vitamin D by sunlight, such as latitude, available UVB radiation throughout the year, skin pigmentation, age, individual behavior, diet and genetic factors. We urgently need to define more closely the interaction between nutrition and genetics in response to vitamin D supplementation. There is a considerable lack of data in both Brazil and UK populations. (Adams and Hewison, 2010; Lanham-New et al, 2012)

Based on the important contribution of sunlight exposure to the production and maintenance of serum 25OHD levels, it would be reasonable to believe that vitamin D deficiency may be a problem restricted to countries situated at higher latitudes. However, several studies from sunny countries have shown that vitamin D deficiency is a more common phenomenon, despite the abundance of sunlight in these locations making it a global health problem. (Lips et al, 2001; Bandeira et al, 2010). Even though Brazil is geographically located such that there is potentially an abundance of sunlight and therefore of vitamin D available throughout the year, there is still evidence of sub-optimal levels of vitamin D in the population. A cross-sectional study conducted in Rio de Janeiro, (latitude 22 ° S), has shown a high incidence of inadequate serum 25 (OH) D (68.3 %) in healthy adults (Russo et al., 2009). Much of the UV in sunlight is absorbed by clouds, ozone and other forms of atmospheric pollution. In higher latitudes as the distance from the equator increases, zenith angle is increased during winter months and consequently, the UV radiation reaching the earth’s surface is reduced, and therefore the cutaneous production of vitamin D3 is also reduced. (Lips et al 2006; Wacker and Holick, 2013). Thus, the cutaneous synthesis of vitamin D is expected to be greater in low- latitude regions due to greater exposure to UVB radiation. In the UK, there is no UV radiation of the appropriate wavelength (280mm-310mm) from the end of October to the end of March and for the remaining months of the year, 60% of the effective UV radiation occurs between 11.00am and 3.00pm. Moreover, studies have shown the influence of skin pigmentation in reducing the UVB-mediated synthesis of vitamin D. Greater quantification of the effect of sunlight exposure on vitamin D status is urgently required. Therefore, we wish to investigate the Brazilian population, suspecting that we could be underestimating the level of insufficiency or deficiency of vitamin D in this population, due to the false impression that the level of solar radiation would suffice for people living in sunny countries.

Recent studies have suggested that genetic variation could be a reasonable explanation to the considerable differences on vitamin D levels among population independently of latitude and sunlight exposure. (Valdivielso 2004) Heritability of vitamin D levels has been reported to range from 28.8% to 68.9% in an adolescent twin study and up to 80% in a genome-wide linkage scan. (Uнтерлиденген 2004, Santos 2012). Identifying genetic variants in the form of single nucleotide polymorphisms (SNPs) has been one of the key methods for identifying genetic variants associated with vitamin D levels. The data would be helpful for identifying those who may be at risk of deficiency and targeting treatment to reduce the impact of vitamin D deficiency. The study will undertake a Genome-wide analysis of leukocyte gene expression and its correlation with differences in vitamin D status. The Vitamin D Receptor (VDR) is a nuclear transcription factor which controls expression of a wide range of genes, including several that encode cytochrome P450 enzymes and cytokines. Human leukocytes are known to express the VDR-encoding gene and it is therefore envisaged that differences in vitamin D responses of individuals could be reflected by differences in leukocyte gene expression. Therefore we propose to analyse genome-wide transcriptomic expression within leukocytes of selected participants in order to associate specific signal transduction and metabolic pathways to respective vitamin D responses. Circulating 25(OH)D concentrations have been shown to be strongly related to SNPs located on exons of the vitamin D binding protein gene: i.e. minor SNP variants of DBP-1 (rs7041, GG®TT (17.5%) and DBP-2 (rs4588, CC®AA (9%), resulting in 10-13% lower circulating 25OHD levels.
Our results will firstly enable determination of how important (as a % contribution) diet, sunlight exposure and genetic factors are to vitamin D status when directly comparing, using the same methodology, same ethnicity populations living in a Southern Hemisphere (where there is abundant sunlight exposure) and in a Northern Hemisphere (where there is only sufficient sunlight exposure during the months April to September).

The data will provide both countries with key data on whether there should be consideration of further revisions to dietary recommendations for vitamin D in adult populations. This study provides good ‘value for money’ since it draws upon previously collected data in the UK and enables a strong study design to be conducted in the two Countries, simultaneously. The data will be of great interest to the field of Nutritional Sciences by the provision and formulation of data, within a short period of time, examining the interaction of diet and the environment on vitamin D status and its functional consequences. The ‘systems level’ approach will enable us to identify differences in gene expression and whether this explains why some individuals are ‘good’ responders or ‘poor’ responders to vitamin D supplementation (Wang et al, 2010). This has never been done before in the field, by examining two population groups living in different countries but following identical study designs. Also, to our knowledge, this is the first randomized control trial to analyse the effect of vitamin D supplementation in Brazilian women, either living in the UK or in Brazil.

2.0 Objectives & Design

2.1 Primary Objectives: To examine the response (in vitamin D status) to vitamin D supplementation and sunlight exposure, and its influencing factors within and between Brazilian female adults living in the UK and Brazilian female adults living in Brazil.

2.2 Secondary Objectives

(i) Analyze the difference regarding time and intensity of sun exposure between Brazilian women living in Brazil and Brazilian women living in the UK.
(ii) Determine the prevalence of inadequate dietary Vitamin D intake in these women.
(iii) Determine the prevalence of insufficient/deficient levels of vitamin D in these women.
(iv) To elucidate the association between Vitamin D status and markers of calcium of calcium metabolism.
(v) Examine the influence of latitude on vitamin D optimal levels.
(vi) Analyze the influence of skin pigmentation on vitamin D optimal levels.
(vii) Analyze if response to Vitamin D supplementation is dependent on initial vitamin D levels.
(viii) Investigate the genetic and enzymatic mechanisms underlying the adolescents’ response to vitamin D supplementation via genotyping for polymorphisms related to vitamin D metabolism and comparing the high and low responders to supplementation (Genetic Sub-Study).

2.3 Hypothesis

The study hypothesis that, during winter, vitamin D supplementation is required to obtain Vitamin D optimal levels in Brazilian women residing in the UK as well as Brazilian women residing in Brazil and that this response is dependent on the initial levels, being also influenced by sunlight exposure, skin pigmentation and polymorphisms of the vitamin D receptor gene.

H1: Dietary intakes of vitamin D are too low and sunlight exposure is insufficient for optimal vitamin D status in Brazilians living in the UK and Brazilians living in Brazil.

H2: The response of subjects to vitamin D supplementation is dependent on baseline 25OHD levels.

H3: Response to supplementation is influenced by sun light exposure.
H4: Skin pigmentation is an influential factor on vitamin D optimal levels.

H5: There will be strong genetic influences on 25OHD changes in response to vitamin D supplementation; these will be similar in both groups.

3.0 Experimental Design

Two controlled, randomized, double-blind clinical trials will be developed and undertaken (one in Brazil and the other in the UK) with an intervention period of 12 weeks.

A questionnaire to screen for the relevant inclusion and exclusion criteria (see section A) will be administered in order to select 80 Brazilian female subjects, aged 20 to 59 years, in each of the two countries. The women selected will be randomly divided into two groups: Placebo Group and Supplemented Group, the latter will receive 600UI UI of vitamin D.

The first clinical trial will run in the UK from October 2016 to March 2017 (autumn-winter) and then the second will run in Brazil from April 2017 to September 2017 (autumn-winter). After the analysis of the effect of vitamin D supplementation compared to placebo, the results obtained in the supplemented group will also be analysed according to the genotypes for SNPs in the VDR gene.

We have chosen 12 weeks (3 months) as the supplementation period as we consider this to be ample time for 25OHD levels to rise (half-life of 25OHD is 3 weeks) and this has been demonstrated to be an effective timeframe for supplementation of vitamin D in previous studies.

We have chosen 600IU to enable the study to be relevant to the new IOM US recommendations for vitamin D, which are currently the reference document used in Brazil, even though the Brazilian nutritional table remains with the daily intake recommendation of 200 IU, which was the IOM recommendation in 1997. Compliance will be checked by compliance interviews during the trial and by empty packaging count.

We will genotype vitamin D related genes in DNA isolated from the study blood samples, DNA will be extracted and vitamin D polymorphisms will be determined by our genetic labs. These will include known candidate variants (VDR, vitamin D binding protein, CYP2R1, CYP27A1, CYP27B1, and CYP24A1), as well as variants identified by the ongoing genome-wide association analyses on 25OHD. We will construct a genetic risk score based on these polymorphisms. The Genome-wide expression profiling of RNA from leukocytes will focus on 48 participants (each at two sampling time points) that represent equally both groups, encompassing the best and worst supplementation-responders in each group. We will identify the complete spectrum of genes that are, by statistical criteria, either significantly up-or down-regulated (i.e. differentially expressed) between groups. The major comparison will be between the ‘good’ and ‘poor’ responders in each group; gene expression prior to supplementation will be compared to after supplementation for each subject analysed.

3.1 Sample Size and randomisation

A total of 80 subjects (at 90% power) are required for recruitment into the 600 IU vitamin D group vs placebo group for the RCT to be conducted in the UK and a total of 80 subjects (at 90% power) are required for recruitment into the 600 IU vitamin D group vs. placebo group for the RCT to be conducted in Brazil. This will enable us to detect a 0.6 SD size effect at 90% power in serum 25OHD levels between placebo and 600 IU in UK-dwelling Brazilian populations and Brazilians living in Brazil. These study numbers of 40 subjects for the vitamin D group and n 40 for the placebo group includes a 20% drop-out rate factored in.

3.2 Recruitment
Participants in the UK will be recruited through advertisement among local Brazilian societies/groups within Surrey and London. Informative posters will be positioned around the University of Surrey and commercial centres (with a focus on Brazilian themed places), with permission of the owner.

As recruitment method, we will ask, after clearly explaining our intentions of divulging the study, Brazilian institutions in the UK, such as the Brazilian Consulate and the Brazilian Researchers Association (ABEP-UK) to circulate a recruitment letter to their contact list of Brazilians living in the UK. If we succeed in this arrangement, this letter will be send directly to those in the lists by the institution, therefore we will not need to have access to people’s contact details.

Social media sites such as Facebook will be used to publicise the study: the poster or flyer will be inserted as a photo with permission of the administrator of the Facebook pages.

Participants in Brazil will be recruited from the general public within the city of Goiânia through posters to be positioned around the Federal University of Goiás and commercial centres, with permission of the owner.

Social media sites such as Facebook will be used to publicise the study: the poster or flyer will be inserted as a photo with permission of the administrator of the Facebook pages.

The recruitment end date for the UK subjects will be 14th December 2016. Recruitment end date for the Brazilian subjects will be the 13th June 2017.

Subjects will be reimbursed for their travel expenses.

### 3.3 Selection & Withdrawal of Participants

#### 3.3.1 Inclusion Criteria

- Brazilian nationality
- Female
- Aged 20-59 years

#### 3.3.2 Exclusion Criteria

- Currently receiving treatment for medical conditions that are likely to affect vitamin D metabolism (osteoarthritis therapy, anti-estrogens treatment, antiepileptic drugs, breast-cancer treatment)
- Hypercalcaemia (>2.5mmol/L) – assessed and excluded at baseline
- Regular use of sun-beds
- Having a holiday trip one month prior to commencing the study or plans for a holiday trip for more than 4 weeks out of the country of residence within the study period.
• Use of vitamin supplements containing vitamin D (if the prospective participants agrees to stop Vitamin D supplementation to join the study, a wash-out period of 8 weeks prior to commencing the trial would be acceptable).
• Pregnant or planning a pregnancy during the study period.

If a participant is subsequently found to be ineligible for the study their screening questionnaire will be destroyed due to the questionnaire containing sensitive information.

3.3.3 Withdrawal

All participants will be notified during the consenting process that they are free to withdraw from the trial at any time, without giving a reason.

Participants will be withdrawn from the trial by the Principal Investigator if:

1. The participant develops a medical condition or becomes pregnant either prior to entering the study or during, which may adversely affect the outcome of the study.
2. It is clearly demonstrated that the participant is non-compliant completing study activities and the control procedures requested of them.
3. A participant suffers an adverse event, which will be reviewed and recorded at each visit and at each phone call, following the immediate discontinuation of the participant during the visit, if necessary.

All data prior to subject withdrawal will be used in analysis; unless the participant specifically requests that their data is not to be used. Withdrawn participants will not be replaced as an anticipated drop-out rate of 20% has been accounted for in the recruitment targets.

4.0 Trial Procedures

4.1 Trial visit activities

During this study the subjects will be asked to visit the Clinical Investigation Unit, FHMS, University of Surrey in the UK or the Research Clinic, Faculty of Nutrition, Federal University of Goias in Brazil, on two occasions, at the beginning of the study for baseline measurements and at the conclusion of the study.

We will examine four to eight fasted subjects per study morning.

Trial visits will last approximately 45-60 minutes each and take place in the morning (7am-11am). Participants will be offered refreshments at the end of their appointment.

Consenting and Screening

If participants wish to be screened for participation in the study, they will receive the Participant Information Sheet and then be checked against the study inclusion and exclusion criteria using the ‘Screening Questionnaire’ (Section A), to be administered by a member of the D-SOL Research Team by phone or self-reported by email.

Baseline visit

If eligible, participants will be invited for the baseline visit. At this visit, they will first be given time to discuss the Participant Information Sheet and any questions they may have regarding the study. Informed consent will be discussed and participants will be asked to sign the consent form, and will be offered a copy to keep for themselves.
• Health and Lifestyle questionnaire to be administered by a member of the D-SOL Research Team.
• Anthropometrics and blood pressure measured, and fasted blood sample taken (serum 25OHD levels, 1,25-dihydroxy vitamin D, serum calcium, albumin, parathyroid hormone, C-terminal telopeptide (CTX) ≈25ml) with an additional ≈10 ml for genetic profiling and ≈15 for storage for future measurements of nutritional markers.
• pQCT scan of the non-dominant forearm for UK trial or DEXA scan for Brazil trial.
• Bioelectrical impedance analysis (BIA) for body composition.
• Provision of randomly assigned daily supplement (30 days’ supply), food diaries and dosimeter (to be returned via SAE provided) and sunlight exposure diary to be returned at 12 week visit. Follow-up appointment details arranged (including interim visit telephone appointments and for re-supply of supplement).

Final visit

• Final adverse event/compliance interview completed with investigator.
• Daily outdoor exposure diary received from participant and check for consistency at visit.
• Anthropometrics and blood pressure measured, and blood sample taken (serum 25OHD levels, 1,25-dihydroxy vitamin D, serum calcium, albumin, parathyroid hormone, C-terminal telopeptide (CTX) ≈25ml) with an additional ≈10 ml for genetic profiling and ≈15 for storage for future measurements of nutritional markers.
• Bioelectrical impedance analysis (BIA) for body composition.
• Dosimeter and 4-day food diary received from participant (sent to participant prior to appointment).

DNA profiling procedure - After 12 weeks RCT

• Selection of 48 participant samples encompassing the best and worst supplementation-responders in each group, subject to previous consent form participant. Vitamin D related genes will be genotyped in DNA isolated from the study blood samples, DNA will be extracted and vitamin D polymorphisms will be determined by University of Surrey’s genetic labs.

A trained phlebotomist will take the blood samples required as part of the trial protocol. Medical cover will be available at all times.

Throughout the duration of the trial, the participants will be contacted via telephone on a fortnightly basis to discuss any issues with any adverse event and compliance and to maintain good communication with the participants. (Please see AE reporting form). In the case of a serious adverse event (SAE) this will be recorded (please see SAE reporting form) and we will report it to both the Sponsor (University of Surrey) and the Surrey Ethics Committee.

The final interview will be completed at the final study visit. Participants have also be asked to return any supplements that were missed to confirm compliance.

For University of Surrey participants: A peripheral quantitative computed tomography (pQCT) scan was performed on the participant's non-dominant forearm at the baseline visit, to measure volumetric bone mineral density at the 4% and 66% radial site. This will allow for separate measurements of trabecular vBMD and trabecular area (4% site) and cortical vBMD and cortical area (66% site), as well as strength strain index, a measure of bone strength. pQCT also measures bone geometry alongside bone density. Therefore the muscle cross sectional area can be determined, which is a measure of muscle force, to which bone strength is adapted to. One scan was performed at baseline only and effective exposure doses were between ~1.5-1.8uSv.

For Federal University of Goiás participants: Body composition (absolute and relative amount of lean and fat mass), whole body mineral density and lower spine and femur bone mineral density was measured with the use of DEXA (located at the Nutrition Clinic based at the Federal University of Goias), at baseline only. Two scans were performed at baseline for each participant: one to assess the whole
body mineral density and body composition, and the other to specifically assess fracture risk by scanning the spine and femoral head. Effective exposure doses for these scans are ~8uSv and ~4uSv respectively.

Results of the body composition, vitamin D status and dietary intake from the self-reported food diaries will be made available to the subjects upon request. The results from the blood analysis will be reported to the subject if there are any health concerns raised. If the results are within healthy ranges the participants will not be contacted unless they specifically request for this information. The investigators will not be contacting their GPs if there are any concerns raised in the study however we will stress that they should contact their GP themselves to discuss the results we found.

5.0 Benefits and risks of participants in the D-SOL Study

Results of the body composition, vitamin D status and dietary intake from the self-reported food diaries will be made available to the subjects upon request.

We do not anticipate identifying anything of concern via either the blood tests or bone scan, as we’re not measuring any markers that would actually indicate any serious pathology is occurring. A letter informing that the participant is on the trial will be sent the participant’s GP. A response from the GP will not be required unless the GP had concerns and no results will be sent to them afterwards. If the results are within healthy ranges the participants will not be contacted unless they specifically request for this information. If abnormal results came back for a participant, they will be offered support in approaching the GP, all the information regarding their test results, details on the intervention, etc, will be made available.

Due to the trial being food based, the risk of side-effects is minimal. However, gastrointestinal discomfort may occur following supplement consumption.

A blood sample must be taken at each trial visit, including screening, and due to the nature of the procedure, some light bruising may occur. Occasionally, fainting in some individuals can occur relating to venepuncture. To help reduce the risk of this, participants will have their blood sample taken either whilst they are supine on a bed or reclined on a chair that has the capacity to be adapted quickly to allow the participant to lie supine safely if they do become unwell.

pQCT and DXA scans use a low level of radiation (much lower than standard X-ray examination) to which participants will be exposed. The amount of radiation absorbed from the scan is very small and similar to the radiation we receive from the environment.

Although health problems linked to vitamin D are very rare, there is some evidence that vitamin D supplementation can cause blood calcium levels to go higher than normal in some people who already have high blood calcium levels. Upon commencing the study, all participants will have their blood calcium levels checked to ensure that the vitamin D would be unlikely to cause any problems.

6. Ethics and regulatory approval

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (2008), the principles of GCP and in accordance with The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006.

This protocol and supporting documents will be submitted for review by the University of Surrey Ethics Committee and the Federal University of Goias, Brazil. Annual progress reports and a final report will be submitted to the ethics committees as defined in their respective regulations.
7.0 Study Evaluation and Statistical Analysis

Statistical analysis will be undertaken with support from the University of Surrey statistical department. Data will be checked for normality using appropriate testing. Appropriate parametric/non-parametric analysis will be applied. The database will be stored and analysed using SPSS® version 13.0. The descriptive analysis, including mean ± standard deviation, median and lower limit and higher will be held for all quantitative variables. Data will be checked for normality using appropriate testing. Appropriate parametric/non-parametric analysis will be applied. Student t test or Mann-Whitney test, depending on the distribution of data, will be applied to evaluate differences between supplemented and placebo. For analysis between Brazil and UK groups an ANOVA test will be undertaken. Linear correlation, Pearson or Spearman, will be calculated in accordance with the presence / absence of normal distribution, respectively, between vitamin D intake, sun exposure, skin pigmentation and vitamin D serum concentration. Multiple linear regression analysis will be performed to determine the variants that influence blood concentrations of vitamin D. In order to determine whether supplemental response differs between genotypes, these will be separated into wild homozygotes, heterozygotes and homozygotes for the variant. In this approach, the results will be evaluated with the test ANOVA or Kruskal-Wallis, in cases of variables with normal distribution or not, respectively. To describe the relationship between food intake aspects and features biochemical independent of energy intake, intake values of nutrients of interest will be adjusted to energy in accordance with the method. Significance considered as standard will be 5%.

8.0 Data Handling

The Principal Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

- Participants data will be completely anonymised
- All anonymised data will be stored in a secure location on the University’s servers and on a password-protected computer these will be in line with best practice as recommended in the University of Surrey Research and Information Governance policies.
- All trial data will be stored and archived as indicated by The Medicines for Human Use (Clinical Trials) Amended Regulations 2006.
- When a subject does not meet the inclusion criteria, all questionnaires and collected data will be destroyed.
- As part of the collaboration agreement with the Federal University of Goias, Brazil, all data collected in both countries will be shared between the two institutions.

9.0 Publication policy

The results of the study will be reported and disseminated to the scientific community via peer-reviewed journals and international conferences. The general public will be engaged via the release of results to the local and national media, relevant charities and community networks and an invited talk at the University.

10.0 Finance

Full funding to conduct the D-SOL Study is provided by the Science Without Borders Program through the National Counsel of Technological and Scientific Development of Brazil (CNPq).

11.0 Signatures

Principal Investigator: Miss Marcela Moraes Mendes Date: 17 January 2016
REFERENCES


SECTION A

THE D-SOL STUDY
SCREENING QUESTIONNAIRE

The purpose of this questionnaire is to assess whether you are suitable to take part in the study and that it is safe for you to do so. *We would be grateful if you would answer the following questions, even if you are still not sure if you wish to take part in our study.* Please answer the questions as honestly and accurately as you can and remember there are no right or wrong answers to the questions. Your answers to the questions on this questionnaire will also be kept completely confidential. If you feel uncomfortable answering any of the questions on this questionnaire you do not have to answer the question. Please ask one of the D-SOL Research Team if you would like help answering any of the questions.

**CONTACT DETAILS**

Name: 
DOB: / / Age: Gender: 
Address: 
Contact telephone number: Nationality: 
Mother’s nationality: Father’s nationality: 
Email address: 
Preferred method of contact: Phone Email Post 

**HEALTH AND LIFESTYLE**

Height (cm): Weight (kg): 
Are you currently receiving treatment for any medical conditions? Yes No 
Medical condition: Treatment: 
Are you on any medication prescribed by your GP or any other health care provider? Yes No If yes, please specify: 

1. Do you live in the UK? Yes No 
If yes, please state the date you arrived: / /
2. Please tick all medical conditions that apply:

<table>
<thead>
<tr>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior/present history of coronary heart disease, angina, heart attack or stroke</td>
</tr>
<tr>
<td>Prior/present history of Type 1 and Type 2 Diabetes.</td>
</tr>
<tr>
<td>Prior/present history of Thyroid disease</td>
</tr>
<tr>
<td>Prior/present history of osteoporosis, osteopenia or other musculoskeletal disease</td>
</tr>
<tr>
<td>Prior/present history of haematological disease (except mild anaemia)</td>
</tr>
<tr>
<td>Prior/present history of malignancy</td>
</tr>
<tr>
<td>Prior/present history of a gastrointestinal disorder, such as Crohns Disease, Coeliac Disease or Irritable Bowel Syndrome.</td>
</tr>
<tr>
<td>Prior/present history of liver or kidney disease.</td>
</tr>
</tbody>
</table>

3. Do you have any allergies or food intolerances?  
   If yes, please specify: .................................................................
   ........................................................................................................

4. Do you regularly take vitamin supplements containing vitamin D?  
   If yes please specify:  
   How many months you have been taking the supplements?......................  
   The dose of the supplements (if known): .............................................  
   The brand of the supplements (if known): ...........................................

5. Are you currently on a weight-reducing diet or other dietary restrictions (except vegetarianism)?  
   If yes, please provide details: ...........................................................

6. Have you been abroad on holiday during the past 6 months?  
   If yes, please specify where this was and the month this holiday was taken:  
   Country of visit……………………………………………………..  
   Month/year……………………………………………………..  
   Country of visit……………………………………………………..
   Month/year……………………………………………………..

6. Are you planning any holidays abroad during the next 12 months?  
   If yes, please specify where this visit is planned to be and when this holiday will be taken:  
   Country of visit……………………………………………………..  
   Month/year .................................................................  
   Country of visit……………………………………………………..
   Month/year .................................................................
7. Do you use sunbeds?
   
   If yes, please state how often you use them:
   
   [ ] Once a week
   [ ] Once a month
   [ ] More than 6 times per year
   [ ] Less than 6 times per year
   [ ] Occasionally

8. Are you currently pregnant or planning a pregnancy during the next 12 months?  
   [ ] Yes  [ ] No

9. Are you currently breastfeeding?  
   [ ] Yes  [ ] No

10. Are you in menopause?  
    [ ] Yes  [ ] No

We are planning to see participants in the morning between 8.00am and 12.00 noon. Are there any particular day or days which would be best for you?

Thank you for taking the time to complete this questionnaire.

Participant signature: ___________________________________________  Quest. No: ______
Date: __/__/____
31 August 2016

Dear Miss Moraes Mendes

**UEC ref: UEC/2016/009/FHMS**

**Study Title:** A systems biology approach to the interaction between vitamin D supplementation and sunlight exposure in Brazilian women living in opposite latitudes (The D-SOL Study)

On behalf of the Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the submitted protocol and supporting documentation.

Date of confirmation of ethical opinion: 31 August 2016

The final list of documents reviewed by the Committee is as follows:

<table>
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<tr>
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<td>Questionnaire</td>
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<td>D-SOL invitation letter</td>
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This opinion is given on the understanding that you will comply with the University's Ethical Principles & Procedures for Teaching and Research.
If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University's Ethics Committee.

If you wish to make any amendments to your protocol please address your request to the Secretary of the Ethics Committee and attach any revised documentation.

The Committee will need to be notified of adverse reactions suffered by research participants, and if the study is terminated earlier than expected with reasons. Please be advised that the Ethics Committee is able to audit research to ensure that researchers are abiding by the University requirements and guidelines.

You are asked to note that a further submission to the Ethics Committee will be required in the event that the study is not completed within five years of the above date.

Please inform me when the research has been completed.

Yours sincerely

Miss Rebecca Green
Research Integrity and Governance Co-ordinator, Research & Enterprise Support

Copy to.
Professor Susan Lanham-New, School of Biosciences and Medicine, FHMS
Miss Marcela Moraes Mendes  
School of Biosciences and Medicine  
FHMS

23 September 2016

Dear Miss Moraes Mendes

UEC ref: UEC/2016/009/FHMS Amendment 1

Study Title: A systems biology approach to the interaction between vitamin D supplementation and sunlight exposure in Brazilian women living in opposite latitudes (The D-SOL Study)

I am writing to inform you that the Chairperson, on behalf of the Ethics Committee, has considered the Amendment requested to the above protocol and supports a favourable ethical opinion on the understanding that the University's Ethics Handbook for Teaching and Research is observed. Please be advised that the Ethics Committee is able to audit research to ensure that researchers are abiding by the University requirements and guidelines.

If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University’s Ethics Committee.

Date of confirmation of ethical opinion: 31 August 2016

Date of favourable ethical opinion of amendment to protocol: 23 September 2016

The list of amended documents reviewed and approved by the Chairperson is as follows:-

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Please note: you should only be using the versions of the documents referred to in this letter. If you intend to update these documents, you must notify the University Ethics Committee.
Yours sincerely

Miss Madeleine Blair
Research Integrity and Governance Co-ordinator, Research & Enterprise Support

Copy to. Prof Susan Lanham-New, School of Biosciences and Medicine, FHMS
30 November 2016

Dear Miss Moraes Mendes

**UEC ref: UEC/2016/009/FHMS Amendments 2 and 3**

**Study Title:** A systems biology approach to the interaction between vitamin D supplementation and sunlight exposure in Brazilian women living in opposite latitudes (The D-SOL Study)

I am writing to inform you that the Chairperson, on behalf of the Ethics Committee, has considered the Amendment requested to the above protocol and supports a favourable ethical opinion on the understanding that the University’s Ethics Handbook for Teaching and Research is observed. Please be advised that the Ethics Committee is able to audit research to ensure that researchers are abiding by the University requirements and guidelines.

If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University’s Ethics Committee.

Date of confirmation of ethical opinion: 31 August 2016

Date of favourable ethical opinion of amendment to protocol: 30 November 2016

The list of amended documents reviewed and approved by the Chairperson is as follows:

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Please note: you should only be using the versions of the documents referred to in this letter. If you intend to update these documents, you must notify the University Ethics Committee.

Yours sincerely

![Signature]

Miss Madeleine Blair
Research Integrity and Governance Co-ordinator

Copy to. Prof Susan Lanham-New, School of Biosciences and Medicine, FHMS
INTRODUÇÃO
Ao longo dos últimos 15 anos, a vitamina D tem sido bastante estudada pela comunidade científica. Embora existam muitas questões ainda controversas em relação à concentração ideal de vitamina D sérica e o impacto da ingestão de vitamina D sobre a saúde humana, há um consenso de que a deficiência de vitamina D é um problema de saúde pública em razão de suas implicações em diversas doenças entre elas, a osteopenia, osteomalácia, doenças cardíacas, diabetes tipo I e II, doenças inflamatórias, esclerose múltipla e artrite reumatoide. A vitamina D é derivada de duas fontes: da pele (endógena) e da dieta (exógena). A principal fonte de vitamina D é a exposição da pele aos raios UV-B contidos na luz solar, uma vez que esta é capaz de estimular a conversão do 7-dehidrocolesterol na pele a pré-colecaciferol. Desta forma, considerando a expressiva contribuição da fonte endógena de vitamina D, a sua concentração pode ser influenciada por diversos fatores, a destacar: fatores ambientais.
como a latitude e as condições meteorológicas, atributos pessoais, tais como a pigmentação da pele, idade, vestuário, uso do protetor solar, ambiente de trabalho, atividade física e exposição ao sol e fatores genéticos. Com base na importante contribuição da exposição à luz solar para a produção e manutenção dos níveis séricos de 25(OH)D, seria razoável se constatar que a deficiência de vitamina D é um problema restrito a países situados em latitudes mais altas.

No entanto, vários estudos realizados em países ensolarados, como o Brasil, têm demonstrado que a deficiência de vitamina D é um fenômeno comum, apesar da abundância de luz solar nestes locais, o que evidencia a influência também da dieta e de fatores genéticos sobre o metabolismo da vitamina. Quanto aos fatores dietéticos, o Brasil ainda não possui recomendações de ingestão diária de vitamina D específicas para a sua população. Atualmente, são utilizadas as Dietary Reference Intakes (DRI) do Instituto de Medicina dos Estados Unidos (IOM) de 600UI/dia ou 15g/d de vitamina D. No entanto, essa recomendação tem sido alvo de crítica de especialistas da área, que defendem que este valor seja insuficiente para manter a concentração sérica adequada. Em relação aos fatores genéticos, estudos tem demonstrado que o gene para o receptor da vitamina D (VDR) desempenha um papel importante no metabolismo dessa vitamina. Polimorfismos nesse gene podem potencialmente influenciar a estabilidade do mRNA e, consequentemente a expressão e a concentração sérica dessa vitamina.

Um grupo de Porto Alegre encontrou uma prevalência de 33% de deficiência de vitamina D em adolescentes brasilereiras saudáveis e demonstrou que esta pode estar associada a polimorfismos do gene do receptor específico da vitamina D (VDR). Desta forma, considerando que a ingestão dietética de vitamina D é baixa e o tempo de exposição solar pode ser insuficiente para a manutenção dos níveis adequados de vitamina D em populações do Brasil e do Reino Unido, é de extrema importância avaliar o efeito da suplementação dessa vitamina em populações de mesma etnia, porém residentes em latitudes diferentes de modo a verificar a influência da exposição solar e dos fatores genéticos sobre a resposta a suplementação.

HIPÓTESE

H1: A suplementação de vitamina D é necessária para se obter a concentração sérica ideal tanto em brasileiras residentes no Brasil quanto no Reino Unido.

H2: A ingestão dietética e a exposição à luz solar na população brasileira, tanto em mulheres residentes no Brasil quanto no Reino Unido, é insuficiente para manutenção da concentração sérica adequada de vitamina D.

H3: A resposta a suplementação de vitamina D é dependente dos níveis séricos iniciais.
H4: A resposta à suplementação é influenciada pela incidência de luz solar.

H5: A resposta à suplementação com vitamina D é influenciada por polimorfismos do gene do receptor da vitamina D.

H6: A pigmentação da pele é um fator influente sobre a concentração sérica ideal de vitamina D.

METODOLOGIA

O estudo é um ensaio clínico controlado, randomizado, duplo-cego com duração de 12 semanas. Durante o recrutamento, será realizada a aplicação de um questionário com os fatores de inclusão e exclusão de forma a selecionar 80 indivíduos do sexo feminino, brasileiras, com idade entre 20 e 59 anos em cada um dos países, que concordem com o termo de consentimento livre e esclarecido. Para o cálculo da amostra foi utilizado um poder estatístico de 90% com margem de erro de 3,5%, resultando em uma necessidade de pelo menos 30 participantes em cada grupo para ser possível avaliar alterações significantivas nos níveis séricos de vitamina D entre os grupos. A amostra de 40 indivíduos para o grupo vitamina D e 40 para o grupo placebo inclui uma taxa de abandono de 20%. As 80 mulheres selecionadas em cada país serão divididas em dois grupos: Grupo Placebo e Grupo Suplementado, que receberá 600UI de vitamina D. O primeiro ensaio clínico será executado no Reino Unido de novembro de 2016 a março de 2017 (outono/inverno) e, em seguida, o segundo será executado no Brasil de maio de 2017 a outubro de 2017 (outono/inverno).

O projeto foi propositalmente delineado de forma que possam ser excluídas quaisquer exposições exógenas a raios UVB durante este período no Reino Unido. No Brasil, considerando que as estações do ano não são bem definidas, haverá uma exposição a luz solar o que permitirá avaliar diferenças na resposta a suplementação em função da associação com a exposição solar. Optou-se por 12 semanas (3 meses) como o período de suplementação por estudos anteriores terem mostrado ser um período de tempo eficaz para a suplementação de vitamina D. Já a suplementação com 600IU foi escolhida por ser as recomendações do IOM dos EUA para a vitamina D, que são atualmente as recomendações adotadas no Brasil. A aderência será verificada regularmente por telefone e pessoalmente na última visita, pela contagem das embalagens vazias. Os resultados obtidos nos grupos suplementados também serão analisados de acordo com os genótipos para os seguintes SNPs no gene do VDR: FokI TC (rs10735810), BsmI AG (rs1544410), Apal GT (rs7975232), e Taql CT (rs731236). Serão realizadas coletas de sangue, antropometria e avaliação da pigmentação da pele no início e no final da intervenção (T0 e T12s) e composição corporal e densitometria óssea.
apenas em T0. As amostras de soro (no início do estudo e após 12 semanas) serão identificadas e armazenadas em freezers -80 °C, no laboratório “Nutritional Metabolism” da Universidade de Surrey. As seguintes análises bioquímicas serão realizadas: concentração de 25 (OH) D3, 1,25 (OH) D3, fósforo, cálcio, perfil lipídico, glicose, insulina, albumina, hormônio paratireoide, CTX (telopeptídio C-terminal). A ingestão alimentar dos indivíduos será avaliada por meio de registros alimentares de 4 dias consecutivos no início e no final da intervenção. Para verificação da exposição à luz solar os indivíduos usarão broches dosímetros de exposição a raios UV individuais no começo e no final do período de coleta. Os valores de massa livre de gordura (MLG), o percentual de gordura corpórea (%G) e a densitometria óssea serão avaliados no Laboratório de Investigação em Nutrição Clínica e Esportiva da Faculdade de Nutrição da Universidade Federal de Goiás (UFG), utilizando o método de absorciometria radiológica de feixe duplo (DXA) em equipamento modelo DPX NT. As análises de DNA serão realizadas nos laboratórios de genética da Universidade de Surrey. O DNA genômico será extraído com o auxílio do kit comercial High Pure PCR Template Preparation (Roche®, Mannheim, Alemanha), a partir de leucócitos presentes em sangue periférico. A quantidade de pigmento melanina na pele será mesurada por meio de questionário validado de classificação do fototipo de pele de Fitzpatrick.

**METODOLOGIA DE ANÁLISE DE DADOS:**

O banco de dados será elaborado utilizando o programa SPSS ®, versão 13.0 com dupla entrada para a conferência dos dados por meio do validate. A análise descritiva, incluindo média ± desvio-padrão, mediana e limite inferior e superior serão realizados para todas as variáveis quantitativas. Inicialmente, os dados serão testados quanto a sua distribuição, por meio do teste de Shapiro-Wilk. Teste t de Student ou Mann-Whitney, dependendo da distribuição dos dados serão aplicados para avaliar diferenças entre o grupo suplementado e placebo. Para análise conjunta dos quatro grupos será realizada a ANOVA. Correlações lineares de Pearson ou Spearman serão calculadas de acordo com a presença/ausência de distribuição normal, respectivamente, entre ingestão de vitamina D, exposição ao sol e concentração sérica de vitamina D. A análise de regressão linear múltipla será realizada para determinar as variáveis que mais influenciaram as concentrações sanguíneas de vitamina D. A fim de determinar se a resposta a suplementação difere entre os genótipos, estes serão separados em homozigotos selvagens, heterozigotos e homozigotos para a variante. Nesta abordagem, os resultados serão avaliados com o teste de ANOVA ou Kruskal-Wallis, em casos de variáveis com distribuição normal ou não, respectivamente. Correlações
lineares de Pearson ou Spearman serão calculadas também de acordo com a presença/ausência de distribuição normal, respectivamente. O equilíbrio de Hardy-Weinberg será verificado por meio do teste de Qui-Quadrado com o auxílio da calculadora para marcadores bialélicos. Para descrever a relação entre aspectos do consumo alimentar e características bioquímicas independente da ingestão de energia, os valores de ingestão dos nutrientes de interesse serão ajustados ao valor energético, de acordo com o método residual proposto por Willet, Howe and Kushi. O nível de significância adotado como padrão será 5%.

DESFEOCH PRIMÁRIO:
Concentração sérica de vitamina D.

DESFEOCH SECUNDÁRIO:
Concentração sérica de cálcio, perfil lipídico, glicose, insulina, albumina, hormônio paratireoide, CTX (telopeptídio C-terminal).

CRITÉRIOS DE INCLUSÃO
Sexo feminino, brasileira, com idade entre 20 e 59 anos.

CRITÉRIOS DE EXCLUSÃO
Mulheres em uso de medicamento ou com doenças que possam afetar o metabolismo da vitamina D; uso de suplementos; que utilizaram camas de bronzeamento artificial; mulheres grávidas ou amamentando; mulheres em menopausa ou reposição hormonal.

Objetivo da Pesquisa:

OBJETIVO PRIMÁRIO
Avaliar o efeito da suplementação de vitamina D sobre marcadores do metabolismo ósseo em mulheres adultas brasileiras residentes no Brasil e no Reino Unido.

OBJETIVOS SECUNDÁRIOS
(I) Avaliar a diferença em relação ao tempo e intensidade de exposição ao sol entre mulheres adultas brasileiras que vivem no Brasil e mulheres adultas brasileiras que vivem no Reino Unido.
(II) Determinar a prevalência de ingestão inadequada de vitamina D em mulheres adultas brasileiras residentes no Brasil e no Reino Unido.
(III) Determinar a prevalência de níveis insuficientes / deficientes de vitamina D em mulheres adultas brasileiras residentes no Brasil e no Reino Unido.
(IV) Investigar a influência da pigmentação da pele sobre a concentração de vitamina D em mulheres adultas brasileiras residentes no Brasil e no Reino Unido.
(V) Investigar se a resposta à suplementação de vitamina D é dependente da concentração inicial
dessa vitamina.

(VI) Determinar a influência de fatores genéticos nas respostas a suplementação de vitamina D.

(VII) Avaliar a associação entre níveis séricos de vitamina D e marcadores do metabolismo ósseo.

(VIII) Determinar a influência da latitude na manutenção dos níveis séricos adequados de vitamina D.

Avaliação dos Riscos e Benefícios:

RISCOS:
O risco é mínimo e somente associado ao desconforto da coleta de sangue (inchaço e rubor) e a um possível constrangimento durante a entrevista. No entanto, o participante pode se recusar a responder qualquer uma das perguntas sem que isto lhe traga qualquer prejuízo. Não foram identificados riscos relacionados à ingestão de cápsulas de vitamina D com dosagem 600UI em pesquisas anteriores. Exposição ao raio-X do equipamento DXA é considerada mínima e não acarretará prejuízos a saúde.

BENEFÍCIOS:
Resultados dos exames de composição corporal, densitometria óssea e marcadores do metabolismo ósseo serão disponibilizados aos participantes. Participantes do grupo suplementado terão menor risco de níveis deficientes de vitamina D ao final do estudo. A identificação dos fatores relacionados à manutenção de níveis adequados de vitamina D no sangue em mulheres brasileiras permitirá o desenvolvimento de recomendações e orientações de saúde mais específicas e eficazes para esse grupo populacional.

Comentários e Considerações sobre a Pesquisa:
Respostas ao Parecer Consustanciado CONEP nº 1.905.040 de 03/02/2017.

Ensaio clínico controlado, randomizado, duplo-cego com duração de 12 semanas com 80 indivíduos do sexo feminino, brasileiras, com idade entre 20 e 59 anos em cada um dos países (Brasil e Reino Unido). As 80 mulheres selecionadas em cada país serão divididas em dois grupos: Grupo Placebo e Grupo Suplementado, que receberá 600UI de vitamina D. O primeiro ensaio clínico será executado no Reino Unido de novembro de 2016 a março de 2017 (outono-inverno) e, em seguida, o segundo será executado no Brasil de maio de 2017 a outubro de 2017 (outono-inverno).

Serão coletados dados socioeconômicos, características da pele, de estilo de vida e saúde (por meio de questionários aplicados por entrevistadores treinados), dados antropométricos (peso, altura e circunferência da cintura), de padrão alimentar (por meio de diário alimentar de 4 dias consecutivos), exposição a luz solar (por meio de diário e broches dosímetros de raio-ultravioleta).
Amostras de sangue serão coletadas para análises bioquímicas de marcadores do metabolismo ósseo e para análises genéticas (SNPs no gene do receptor de vitamina D - VDR no grupo suplementado). Para verificação da exposição à luz solar os indivíduos usarão dosímetros de exposição a raios UV individuais no começo e no final do período de coleta. A pigmentação da pele será determinada por meio de questionário de fototipo de pele de Fitzpatrick.

Serão realizadas coletas de sangue e avaliação da composição corporal no início e no final da intervenção (T0 e T12s) e densitometria óssea no início apenas (T0). As análises bioquímicas serão realizadas no Departamento de Medicina da Universidade Imperial College London e no laboratório de genética da Universidade de Surrey, sob a responsabilidade da Dra. Lanham-New (Inglaterra). As amostras de sangue coletadas que não forem utilizadas no estudo serão devidamente descartadas.

A pesquisa será custeada pela taxa de bancada da bolsa de doutorado no exterior concedida pelo Programa Ciências Sem Fronteiras, com apoio do CNPq. Os suplementos e placebos serão fornecidos gratuitamente pela Viridian Nutrition Co. (Registration number: 03750310).

Trata-se de projeto de doutorado de Marcela Moraes Mendes da Faculdade de Nutrição da Universidade Federal de Goiás sob orientação da Dra. Patrícia Borges Botelho.

Considerações sobre os Termos de apresentação obrigatória:
Em resposta ao Parecer Consubstanciado CONEP nº 1.905.040 de 03/02/2017 foram postados em 15/03/2017 os seguintes documentos:
• PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_781859.pdf de 15/03/2017
• Carta_resposta_DSOL.pdf
• Aprovacao_Comite_de_Etica_traduzido.pdf
• Declaracao_de_uso_do_material_biolgico_no_exterior.pdf
• ORCAMENTO_REVISADO.pdf
• TERMO_DE_ACORDO_INTERINSTITUCIONAL_EM_PESQUISA.pdf
• termo_anuencia_traduzido.pdf
• Declaracao_estudos_futuros.pdf
• TERMO_DE_CONSENTIMENTO_LIVRE_E_ESCLARECIDO_REVISADO.pdf
• 2_DSOL_PROTOCOLO_REVISADO.pdf
• Detalhamento_operacional_e_de_infraestrutura_do_Lanucli.pdf
• Detalhamento_operacional_e_infraestrutura_do_laboratorio_da_Universidade_de_Surrey.pdf
• Viridian_Supplements.pdf

Endereço: Prédio da Reitoria Téreo Cx. Postal 131
Bairro: Campus Samambaia CEP: 74.001-970
UF: GO Município: GOIANIA
Telefone: (62)3521-1215 Fax: (62)3521-1163 E-mail: cep.prpi.ufg@gmail.com
Documentos postados na submissão inicial (18/11/2016):

- 18_Aprovacao_conselho_diretor.pdf
- 17_Comprovante_taxa_bancada2.pdf
- 16_Comprovante_taxa_bancada.pdf
- 15_treinamento_pessoal.pdf
- 14_instituicao_coresponsavel.pdf
- 13.COMPROMISSOS_VANTAGENS_PAIS.pdf
- 12_Compromissos_vantagens_sujeitos.pdf
- 11_Aprovacao_Comite_etica_UK.pdf
- 10_Termo_anuencia_University_Surrey.pdf
- 9_Termo_anuencia.pdf
- 8_Termo_compromisso.pdf
- 7_Curriculo_Patricia.pdf
- 6_Curriculo_Susan.pdf
- 5_Curriculo_Marcela.pdf
- 4_TCLE.pdf
- 3.ORCAMENTO.pdf
- 2_DSOL_PROTOCOLO.pdf
- 1_Carta_de_encaminhamento.pdf
- 2_folha_rosto.pdf

Carta Resposta ao atendimento da recomendação da CONEP
Versão do documento da Viridian supplements

Conclusões ou Pendências e Lista de Inadequações:
2. Quanto ao Protocolo de Pesquisa:

2.4.1 -索ita-se apresentar declaração da empresa ratificando a doação dos suplementos e dos placebos para o estudo.

RESPOSTA: Declaração anexada (Arquivo: Viridian supplements).

ANÁLISE: PENDÊNCIA ATENDIDA. Foi inserido na Plataforma Brasil a versão traduzida deste documento.

Verifica-se que a recomendação emanada pela CONEP foi atendida.
Após análise dos documentos postados somos favoráveis à aprovação do presente protocolo de pesquisa, smj deste Comitê.

Considerações Finais a critério do CEP:
Informamos que o Comitê de Ética em Pesquisa/CEP-UFG considera o presente protocolo APROVADO, o mesmo foi considerado em acordo com os princípios éticos vigentes. Reiteramos a importância deste Parecer Consustanciado, e lembramos que o(a) pesquisador(a) responsável deverá encaminhar ao CEP-UFG o Relatório Final baseado na conclusão do estudo e na incidência de publicações decorrentes deste, de acordo com o disposto na Resolução CNS n. 466/12. O prazo para entrega do Relatório é de até 30 dias após o encerramento da pesquisa, prevista para junho de 2018.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

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Endereço:  Prédio da Reitoria Téreo Cx. Postal 131
Bairro:  Campus Samambaia
UF: GO  Município: GOIANIA
CEP:  74.001-970
Telefone:  (62)3521-1215  Fax:  (62)3521-1163  E-mail: cep.prpi.ufg@gmail.com
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Situacao do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

GOIANIA, 12 de Abril de 2017

Assinado por:
João Batista de Souza
(Coordenador)
Título da Pesquisa: Efeito da suplementação com vitamina D e do nível de exposição à luz solar em mulheres brasileiras residentes em latitudes opostas (The D-SOL Study)

Pesquisador: Patrícia Borges Botelho

Área Temática: Genética Humana:
(Haverá envio para o exterior de material genético ou qualquer material biológico humano para obtenção de material genético, salvo nos casos em que houver cooperação com o Governo Brasileiro);
(Haverá armazenamento de material biológico ou dados genéticos humanos no exterior e no País, quando de forma conveniada com instituições estrangeiras ou em instituições comerciais)

Versão: 2

CAAE: 62149516.9.0000.5083

Instituição Proponente: Universidade Federal de Goiás - UFG

Patrocinador Principal: CNPQ

Apresentação do Projeto:

INTRODUÇÃO
Ao longo dos últimos 15 anos, a vitamina D tem sido bastante estudada pela comunidade científica. Embora existam muitas questões ainda controversas em relação à concentração ideal de vitamina D sérica e o impacto da ingestão de vitamina D sobre a saúde humana, há um consenso de que a deficiência de vitamina D é um problema de saúde pública em razão de suas implicações em diversas doenças entre elas, a osteopenia, osteomalacia, doenças cardíacas, diabetes tipo I e II, doenças inflamatórias, esclerose múltipla e artrite reumatóide. A vitamina D é derivada de duas fontes: da pele (endógena) e da dieta (exógena). A principal fonte de vitamina D é a exposição da pele aos raios UV-B contidos na luz solar, uma vez que esta é capaz de estimular a conversão do 7-dehidrocolesterol na pele a pré-colecaciferol. Desta forma, considerando a expressiva contribuição da fonte endógena de vitamina D, a sua concentração pode ser influenciada por diversos fatores, a destacar: fatores ambientais, como a latitude e as condições meteorológicas,
atributos pessoais, tais como a pigmentação da pele, idade, vestuário, uso do protetor solar, ambiente de trabalho, atividade física e exposição ao sol e fatores genéticos. Com base na importante contribuição da exposição à luz solar para a produção e manutenção dos níveis séricos de 25(OH)D, seria razoável se constatar que a deficiência de vitamina D é um problema restrito a países situados em latitudes mais altas. No entanto, vários estudos realizados em países ensolarados, como o Brasil, têm demonstrado que a deficiência de vitamina D é um fenômeno comum, apesar da abundância de luz solar nestes locais, o que evidencia a influência também da dieta e de fatores genéticos sobre o metabolismo da vitamina. Quanto aos fatores dietéticos, o Brasil ainda não possui recomendações de ingestão diária de vitamina D específicas para a sua população. Atualmente, são utilizadas as Dietary Reference Intakes (DRI) do Instituto de Medicina dos Estados Unidos (IOM) de 600UI/dia ou 15g/d de vitamina D. No entanto, essa recomendação tem sido alvo de crítica de especialistas da área, que defendem que este valor seja insuficiente para manter a concentração sérica adequada. Em relação aos fatores genéticos, estudos tem demonstrado que o gene para o receptor da vitamina D (VDR) desempenha um papel importante no metabolismo dessa vitamina. Polimorfismos nesse gene podem potencialmente influenciar a estabilidade do mRNA e, consequentemente, a expressão e a concentração sérica dessa vitamina. Um grupo de Porto Alegre encontrou uma prevalência de 33% de deficiência de vitamina D em adolescentes brasileiras saudáveis e demonstrou que esta pode estar associada a polimorfismos do gene do receptor específico da vitamina D (VDR). Desta forma, considerando que a ingestão dietética de vitamina D é baixa e o tempo de exposição solar pode ser insuficiente para a manutenção dos níveis adequados de vitamina D em populações do Brasil e do Reino Unido, é de extrema importância avaliar o efeito da suplementação dessa vitamina em populações de mesma etnia, porém residentes em latitudes diferentes de modo a verificar a influência da exposição solar e dos fatores genéticos sobre a resposta a suplementação.

HIPÓTESE
H1: A suplementação de vitamina D é necessária para se obter a concentração sérica ideal tanto em brasileiras residentes no Brasil quanto no Reino Unido.
H2: A ingestão dietética e a exposição a luz solar na população brasileira, tanto em mulheres residentes no Brasil quanto no Reino Unido, é insuficiente para manutenção da concentração sérica adequada de vitamina D.
H3: A resposta a suplementação de vitamina D é dependente dos níveis séricos iniciais.
H4: A resposta à suplementação é influenciada pela incidência de luz solar.
H5: A resposta à suplementação com vitamina D é influenciada por polimorfismos do gene do receptor da vitamina D.
H6: A pigmentação da pele é um fator influente sobre a concentração sérica ideal de vitamina D.

METODOLOGIA
O estudo é um ensaio clínico controlado, randomizado, duplo-cego com duração de 12 semanas. Durante o recrutamento, será realizada a aplicação de um questionário com os fatores de inclusão e exclusão de forma a selecionar 80 indivíduos do sexo feminino, brasileiras, com idade entre 20 e 59 anos em cada um dos países, que concordem com o termo de consentimento livre e esclarecido. Para o cálculo da amostra foi utilizado um poder estatístico de 90% com margem de erro de 3,5%, resultando em uma necessidade de pelo menos 30 participantes em cada grupo para ser possível avaliar alterações significativas nos níveis séricos de vitamina D entre os grupos. A amostra de 40 indivíduos para o grupo vitamina D e 40 para o grupo placebo inclui uma taxa de abandono de 20%. As 80 mulheres selecionadas em cada país serão divididas em dois grupos: Grupo Placebo e Grupo Suplementado, que receberá 600UI de vitamina D. O primeiro ensaio clínico será executado no Reino Unido de novembro de 2016 a março de 2017 (outono-inverno) e, em seguida, o segundo será executado no Brasil de maio de 2017 a outubro de 2017 (outono-inverno). O projeto foi propositalmente delineado de forma que possam ser excluídas quaisquer exposições exógenas a raios UVB durante este período no Reino Unido. No Brasil, considerando que as estações do ano não são bem definidas, haverá uma exposição a luz solar que permitirá avaliar diferenças na resposta a suplementação em função da associação com a exposição solar. Optou-se por 12 semanas (3 meses) como o período de suplementação por estudos anteriores terem mostrado ser um período de tempo eficaz para a suplementação de vitamina D. Já a suplementação com 600IU foi escolhida por ser as recomendações do IOM dos EUA para a vitamina D, que são atualmente as recomendações adotadas no Brasil. A aderência será verificada regularmente por telefone e pessoalmente na última visita, pela contagem das embalagens vazias. Os resultados obtidos nos grupos suplementados também serão analisados de acordo com os genótipos para os seguintes SNPs no gene do VDR: FokI TC (rs10735810), Bsml AG (rs1544410), Apal GT (rs7975232), e Taql CT (rs731236). Serão realizadas coletas de sangue, antropometria e avaliação da pigmentação da pele no início e no final da intervenção (T0 e T12s) e composição corporal e densitometria óssea apenas em T0. As amostras de soro (no início do estudo e após 12 semanas) serão identificadas e armazenadas em freezers -80 º C, no laboratório “Nutritional...
Comissão Nacional de Ética em Pesquisa

Continuação do Parecer: 1.972.029

Metabolism* da Universidade de Surrey. As seguintes análises bioquímicas serão realizadas: concentração de 25 (OH) D3, 1,25 (OH) D3, fósforo, cálcio, perfil lipídico, glicose, insulina, albumina, hormônio paratireoide, CTX (telopeptídio C-terminal). A ingestão alimentar dos indivíduos será avaliada por meio de registros alimentares de 4 dias consecutivos no início e no final da intervenção. Para verificação da exposição à luz solar os indivíduos usarão broches dosímetros de exposição a raios UV individuais no começo e no final do período de coleta. Os valores de massa livre de gordura (MLG), o percentual de gordura corpórea (%G) e a densitometria óssea serão avaliados no Laboratório de Investigação em Nutrição Clínica e Esportiva da Faculdade de Nutrição da Universidade Federal de Goiás (UFG), utilizando o método de absorciometria radiológica de feixe duplo (DXA) em equipamento modelo DPX NT. As análises de DNA serão realizadas nos laboratórios de genética da Universidade de Surrey. O DNA genômico será extraído com o auxílio do kit comercial High Pure PCR Template Preparation (Roche®, Mannheim, Alemmanha), a partir de leucócitos presentes em sangue periférico. A quantidade de pigmento melanina na pele será medida por meio de questionário validado de classificação do fototipo de pele de Fitzpatrick.

METODOLOGIA DE ANÁLISE DE DADOS:
O banco de dados será elaborado utilizando o programa SPSS®, versão 13.0 com dupla entrada para a conferência dos dados por meio do validate. A análise descritiva, incluindo média ± desvio-padrão, mediana e limite inferior e superior serão realizadas para todas as variáveis quantitativas. Inicialmente, os dados serão testados quanto a sua distribuição, por meio do teste de Shapiro-Wilk. Teste t de Student ou Mann-Whitney, dependendo da distribuição dos dados serão aplicados para avaliar diferenças entre o grupo suplementado e placebo. Para análise conjunta dos quatro grupos será realizada a ANOVA. Correlações lineares de Pearson ou Spearman serão calculadas de acordo com a presença/ausência de distribuição normal, respectivamente, entre ingestão de vitamina D, exposição ao sol e concentração sérica de vitamina D. A análise de regressão linear múltipla será realizada para determinar as variáveis que mais influenciaram as concentrações sanguíneas de vitamina D. A fim de determinar se a resposta a suplementação difere entre os genótipos, estes serão separados em homozigotos selvagens, heterozigotos e homozigotos para a variante. Nesta abordagem, os resultados serão avaliados com o teste de ANOVA ou Kruskal-Wallis, em casos de variáveis com distribuição normal ou não, respectivamente. Correlações lineares de Pearson ou Spearman serão calculadas também de acordo com a presença/ausência de distribuição normal, respectivamente. O equilíbrio de Hardy-Weinberg será verificado por meio da
teste de Qui-Quadrado com o auxílio da calculadora para marcadores bialélicos. Para descrever a relação entre aspectos do consumo alimentar e características bioquímicas independente da ingestão de energia, os valores de ingestão dos nutrientes de interesse serão ajustados ao valor energético, de acordo com o método residual proposto por Willet, Howe and Kushi. O nível de significância adotado como padrão será 5%.

DESFECHO PRIMÁRIO:
Concentração sérica de vitamina D.

DESFECHO SECUNDÁRIO:
Concentração sérica de cálcio, perfil lipídico, glicose, insulina, albumina, hormônio paratireoide, CTX (telopeptídio C-terminal).

CRITÉRIOS DE INCLUSÃO
Sexo feminino, brasileira, com idade entre 20 e 59 anos.

CRITÉRIOS DE EXCLUSÃO
Mulheres em uso de medicamento ou com doenças que possam afetar o metabolismo da vitamina D; uso de suplementos; que utilizaram camas de bronzeamento artificial; mulheres grávidas ou amamentando; mulheres em menopausa ou reposição hormonal.

Objetivo da Pesquisa:

OBJETIVO PRIMÁRIO
Avaliar o efeito da suplementação de vitamina D sobre marcadores do metabolismo ósseo em mulheres adultas brasileiras residentes no Brasil e no Reino Unido.

OBJETIVOS SECUNDÁRIOS
(I) Avaliar a diferença em relação ao tempo e intensidade de exposição ao sol entre mulheres adultas brasileiras que vivem no Brasil e mulheres adultas brasileiras que vivem no Reino Unido.
(II) Determinar a prevalência de ingestão inadequada de vitamina D em mulheres adultas brasileiras residentes no Brasil e no Reino Unido.
(III) Determinar a prevalência de níveis insuficientes / deficientes de vitamina D em mulheres adultas brasileiras residentes no Brasil e no Reino Unido.

(IV) Investigar a influência da pigmentação da pele sobre a concentração de vitamina D em mulheres adultas brasileiras residentes no Brasil e no Reino Unido.

(V) Investigar se a resposta à suplementação de vitamina D é dependente da concentração inicial dessa vitamina.

(VI) Determinar a influência de fatores genéticos nas respostas a suplementação de vitamina D.

(VII) Avaliar a associação entre níveis séricos de vitamina D e marcadores do metabolismo ósseo.

(VIII) Determinar a influência da latitude na manutenção dos níveis séricos adequados de vitamina D.

Avaliação dos Riscos e Benefícios:

RISCOS:
O risco é mínimo e somente associado ao desconforto da coleta de sangue (inchaço e rubor) e a um possível constrangimento durante a entrevista. No entanto, o participante pode se recusar a responder qualquer uma das perguntas sem que isto lhe traga qualquer prejuízo. Não foram identificados riscos relacionados à ingestão de cápsulas de vitamina D com dosagem 600UI em pesquisas anteriores. Exposição ao raio-X do equipamento DXA é considerada mínima e não acarreta prejuízos a saúde.

BENEFÍCIOS:
Resultados dos exames de composição corporal, densitometria óssea e marcadores do metabolismo ósseo serão disponibilizados aos participantes. Participantes do grupo suplementado terão menor risco de níveis deficientes de vitamina D ao final do estudo. A identificação dos fatores relacionados à manutenção de níveis adequados de vitamina D no sangue em mulheres brasileiras permitirá o desenvolvimento de recomendações e orientações de saúde mais específicas e eficazes para esse grupo populacional.

Comentários e Considerações sobre a Pesquisa:
Respostas ao Parecer Consubstanciado CONEP nº 1.905.040 de 03/02/2017.

Ensaio clínico controlado, randomizado, duplo-cego com duração de 12 semanas com 80 participantes.
indivíduos do sexo feminino, brasileiras, com idade entre 20 e 59 anos em cada um dos países (Brasil e Reino Unido). As 80 mulheres selecionadas em cada país serão divididas em dois grupos: Grupo Placebo e Grupo Suplementado, que receberá 600UI de vitamina D. O primeiro ensaio clínico será executado no Reino Unido de novembro de 2016 a março de 2017 (outono-inverno) e, em seguida, o segundo será executado no Brasil de maio de 2017 a outubro de 2017 (outono-inverno).

Serão coletados dados socioeconômicos, características da pele, de estilo de vida e saúde (por meio de questionários aplicados por entrevistadores treinados), dados antropométricos (peso, altura e circunferência da cintura), de padrão alimentar (por meio de diário alimentar de 4 dias consecutivos), exposição a luz solar (por meio de diário e broches dosímetros de raio-ultravioleta). Amostras de sangue serão coletadas para análises bioquímicas de marcadores do metabolismo ósseo e para análises genéticas (SNPs no gene do receptor de vitamina D - VDR no grupo suplementado).

Para verificação da exposição à luz solar os indivíduos usarão dosímetros de exposição a raios UV individuais no começo e no final do período de coleta. A pigmentação da pele será determinada por meio de questionário de fototipo de pele de Fitzpatrick.

Serão realizadas coletas de sangue e avaliação da composição corporal no início e no final da intervenção (T0 e T12s) e densitometria óssea no início apenas (T0). As análises bioquímicas serão realizadas no Departamento de Medicina da Universidade Imperial College London e no laboratório de genética da Universidade de Surrey, sob a responsabilidade da Dra. Lanham-New (Inglaterra). As amostras de sangue coletadas que não forem utilizadas no estudo serão devidamente descartadas.

A pesquisa será custeada pela taxa de bancada da bolsa de doutorado no exterior concedida pelo Programa Ciências Sem Fronteiras, com apoio do CNPq. Os suplementos e placebos serão fornecidos gratuitamente pela Viridian Nutrition Co. (Registration number: 03750310).

Trata-se de projeto de doutorado de Marcela Moraes Mendes da Faculdade de Nutrição da Universidade Federal de Goiás sob orientação da Dra. Patrícia Borges Botelho.
Considerações sobre os Termos de apresentação obrigatória:

Em resposta ao Parecer Consubstanciado CONEP nº 1.905.040 de 03/02/2017 foram postados em 15/03/2017 os seguintes documentos:

- PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_781859.pdf de 15/03/2017
- Carta_resposta_DSOL.pdf
- Aprovacao_Comite_de_Etica_traduzido.pdf
- Declaracao_de_uso_do_material_biologico_no_exterior.pdf
- ORCAMENTO_REVISADO.pdf
- TERMO DE ACORDO INTERINSTITUCIONAL EM PESQUISA.pdf
- termo_anuencia_traduzido.pdf
- Declaracao_estudos_futuros.pdf
- TERMO DE CONSENTIMENTO_LIVRE E ESCLARECIDO_REVISADO.pdf
- 2_DSOL_PROTOCOLO_REVISADO.pdf
- Detalhamento_operacional_e_de_infraestrutura_do_Lanucli.pdf
- Detalhamento_operacional_e_infraestrutura_do_laboratorio_da_University_of_Surrey.pdf
- Viridian_Supplements.pdf

Documentos postados na submissão inicial (18/11/2016):

- 18_Aprovacao_conselho_diretor.pdf
- 17_Comprovante_taxa_bancada2.pdf
- 16_Comprovante_taxa_bancada.pdf
- 15_treinamento_pessoal.pdf
- 14_instituicao_coresponsavel.pdf
- 13_COMPROMISSOS_VANTAGENS_PAIS.pdf
- 12_Compromissos_vantagens_sujeitos.pdf
- 11_Aprovacao_Comite_etica_UK.pdf
- 10_Termo_anuencia_University_Surrey.pdf
- 9_Termo_anuencia.pdf
- 8_Termo_compromisso.pdf
- 7_Curriculo_Patricia.pdf
- 6_Curriculo_Susan.pdf
- 5_Curriculo_Marcela.pdf
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Recomendações:
Verificar item "Conclusões ou Pendências e Lista de Inadequações".

Conclusões ou Pendências e Lista de Inadequações:
Análise de respostas ao Parecer Consustanciado CONEP nº 1.905.040 de 03/02/2017:

1. Quanto aos arquivos "10_Termo_anuencia_University_Surrey.pdf" e "11_Aprovacao_Comite_etica_UK.pdf": Solicita-se apresentar, além das versões originais, as versões traduzidas para o Português.

RESPONSA: As versões traduzidas para o Português dos documentos: 10_Termo_anuencia_University_Surrey.pdf (Arquivo: Termo anuência traduzido) e 11_Aprovacao_Comite_etica_UK.pdf (Arquivo: Aprovação comitê de ética traduzido) foram anexadas. No entanto, não consta as assinaturas visto que os órgãos competentes de Surrey não assinam documentos em uma língua que não seja a original.

ANÁLISE: PENDÊNCIA ATENDIDA.

2. Quanto ao Protocolo de Pesquisa:

2.1 Quanto ao Protocolo de Pesquisa: No item "Riscos" do documento "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_781859.pdf", lê-se: "O risco é mínimo e somente associado ao desconforto da coleta de sangue (inchaço e rubor) e a um possível constrangimento durante a entrevista. No entanto, o participante pode se recusar a responder qualquer uma das perguntas sem que isto lhe traga qualquer prejuízo. Não foram identificados riscos relacionados à ingestão de cápsulas de vitamina D com dosagem 600UI em pesquisas anteriores. Exposição ao raio-X do equipamento DXA é considerada mínima e não acarreta prejuízos à saúde.". Solicita-se
adequação informando os potenciais riscos associados ao uso da vitamina D e/ou outros riscos também associados à pesquisa explicitando ainda as providências e as cautelas a serem tomadas para evitar que ocorram os riscos. Solicita-se ainda adequação do projeto detalhado.

RESPOSTA: O texto foi adequado conforme solicitação, explicitando os riscos e a forma como serão minimizados. Ver documento: TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE) REVISADO, página 5 e 6 e no projeto detalhado “2_DSOLPROTOCOLO REVISADO.pdf” no item acrescentado denominado “5.12 DESCONFORTOS E RISCOS ESPERADOS”, na página 23 e no APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 5 e 6. ANÁLISE: PENDÊNCIA ATENDIDA.

2.2. No item "BENEFÍCIOS" do documento "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_781859.pdf", lê-se: "Resultados dos exames de composição corporal, densitometria óssea e marcadores do metabolismo ósseo serão disponibilizados aos participantes. Participantes do grupo suplementado terão menor risco de níveis deficientes de vitamina D ao final do estudo. A identificação dos fatores relacionados à manutenção de níveis adequados de vitamina D no sangue em mulheres brasileiras permitirá o desenvolvimento de recomendações e orientações de saúde mais específicas e eficazes para esse grupo populacional.". Solicita-se exclusão do trecho "Resultados dos exames de composição corporal, densitometria óssea e marcadores do metabolismo ósseo serão disponibilizados aos participantes" por se tratar de um direito do participante de pesquisa (e não benefício). Caso o estudo não antecipe qualquer benefício direto ao participante, essa informação deve constar no arquivo da Plataforma Brasil e no projeto detalhado. Solicitam-se adequações. RESPOSTA: O texto foi adequado conforme solicitação na plataforma Brasil e no documento TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE) REVISADO, página 6, bem como no projeto detalhado “2_DSOLPROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 6. ANÁLISE: PENDÊNCIA ATENDIDA.

2.3 No item “Países de Recrutamento” do documento "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_781859.pdf" lê-se “País: BRASIL/ Nº de participantes da pesquisa: 80”. Considerando que o protocolo de pesquisa prevê “selecionar 80 indivíduos do sexo feminino, brasileiras, com idade entre 20 e 59 anos em cada um dos países”, solicita-se adequação informando o número de participantes brasileiras que também serão recrutadas no Reino Unido.
RESPOSTA: Considerando que o recrutamento dos participantes no Reino Unido já aconteceu em Outubro a Dezembro de 2016, não foi possível acrescentar o recrutamento com data passada nas informações básicas do projeto na Plataforma Brasil.
ANÁLISE: PENDÊNCIA ATENDIDA.

2.4 - No "APÊNDICE 8 - ORÇAMENTO FINANCEIRO DETALHADO E REMUNERAÇÃO DO PESquisador" lê-se: "Os suplementos e placebos serão fornecidos pela Viridian Nutrition Co.(Registration number: 03750310) gratuitamente."

2.4.1 - Solicita-se apresentar declaração da empresa ratificando a doação dos suplementos e dos placebos para o estudo.
RESPOSTA: Declaração anexada (Arquivo: Viridian supplements).
ANÁLISE: PENDÊNCIA PARCIALMENTE ATENDIDA. Solicita-se submeter na Plataforma Brasil a versão traduzida deste documento.

2.4.2 - Solicita-se acrescentar o valor da doação dos suplementos e placebos no orçamento financeiro do estudo.
RESPOSTA: Foi acrescentado o valor da doação dos suplementos e placebos no orçamento financeiro do estudo no documento ORÇAMENTO REVISADO e no projeto detalhado "2_DSOL_PROTOCOLO REVISADO.pdf", APÊNDICE 8 - ORÇAMENTO FINANCEIRO DETALHADO E REMUNERAÇÃO DO PESquisador.
ANÁLISE: PENDÊNCIA ATENDIDA.

2.5 - Solicita-se esclarecer se o uso da vitamina D conforme a proposição do estudo deve ser constante ou se há um prazo delimitado para o uso. Caso seja necessário o uso constante da vitamina D, solicita-se esclarecer como ocorrerá o fornecimento de suplemento pós-estudo.
RESPOSTA: Conforme o item III.3.d. deve-se assegurar a todos os participantes ao final do estudo, por parte do patrocinador, acesso gratuito e por tempo indeterminado, aos melhores métodos profiláticos, diagnósticos e terapêuticos que se demonstraram eficazes. Ainda, o acesso também deve ser garantido no intervalo entre o término da participação individual e o final do estudo, podendo, nesse caso, esta garantia ser dada por meio de estudo de extensão, de acordo com análise devidamente justificada do médico assistente do participante. O uso da vitamina D conforme a proposição do estudo tem um prazo delimitado de 12 semanas (período total de

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intervenção do estudo), não havendo necessidade de uso constante.

ANÁLISE: PENDÊNCIA ATENDIDA.

2.6 - No projeto detalhado foi informado que o recrutamento dos voluntários será realizado nas dependências da UFG por meio do convite a alunos e funcionários que tenham interesse em participar e que apresentem os critérios de elegibilidade estabelecidos. ALÉM DISSO, SERÁ REALIZADA A DIVULGAÇÃO DA PESQUISA PELAS REDES SOCIAIS. Durante o recrutamento, será realizada a aplicação de um questionário. Diante do exposto:

2.6.1 - Solicita-se esclarecer a forma de recrutamento dos participantes por meio da rede social e, caso necessário, solicita-se apresentação do texto para análise ética.

RESPOSTA: Foi acrescentada a informação de recrutamento solicitada no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, item 5. Delineamento do Estudo e Metodologia, página 15. O recrutamento nas redes sociais será realizado por meio de postagem na página oficial da FANUT-UFG com texto convite e informações básicas para contato, no caso de interesse. Segue texto para análise ética: “Projeto: Ingestão de vitamina D e exposição a luz solar e sua relação com os níveis séricos de vitamina D em mulheres brasileiras residentes em latitudes opostas – The D-SOL Study. Se você é mulher, tem entre 20 e 59 anos e não iniciou a menopausa, participe da nossa pesquisa! Exames a serem realizados: + Composição corporal (% de gordura) + Exame de sangue (vitamina D, cálcio, hormônio da paratireoide); + Perfil genético de genes ligados a concentração de vitamina D no sangue; + Composição óssea Caso participe dessa pesquisa, você estará ajudando a ciência a entender melhor como a vitamina D age no nosso corpo e qual a importância dela para a saúde dos ossos! - Sua participação requer apenas 2 encontros e a ingestão diária de vitamina D (ou placebo) por 3 meses. - Resultados dos exames serão disponibilizados individualmente para os participantes. Para participar ou para ter mais informações entre em contato: Marcela Mendes (62) 81036716 Ou dsol@surrey.ac.uk Esta pesquisa foi aprovada pelos Comitês de Ética da Universidade Federal de Goiás e da Universidade de Surrey.

ANÁLISE: PENDÊNCIA ATENDIDA.

2.6.2 - Solicita-se esclarecer a forma de recrutamento e atendimento das participantes brasileiras na Inglaterra.

RESPOSTA: Foi acrescentada a informação de recrutamento solicitada no projeto detalhado
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ANÁLISE: PENDÊNCIA ATENDIDA.

2.7 - No documento “2_DSOL_PROTOCOLO.pdf”, postado em 18/11/2016, na página 18 de 52, lê-se “Dosímetros são broches pequenos os quais podem ser lidos por um espectrofotômetro a 320nm, antes e após uso [...].” Solicita-se apresentar as especificações técnicas do produto citado no trecho.

RESPOSTA: Foram acrescentadas as especificações técnicas do produto dosímetro no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, item 5.5 Avaliação da Exposição Solar, página 19, conforme solicitado.

ANÁLISE: PENDÊNCIA ATENDIDA.

3 - Quanto à constituição de biorrepositório, a análise do Protocolo de Pesquisa indica que haverá formação de biorrepositório. Assim sendo todos os aspectos relativos à formação de biorrepositório ao longo da execução de uma pesquisa deverão ser seguidos conforme explicitado na Resolução CNS 441/11 e Portaria MS 2201/11, ainda que o armazenamento do material biológico seja temporário sem previsão de uso futuro. Solicita-se uma revisão das referidas normativas e apresentar:

3.1 - Justificativa quanto à necessidade e oportunidade para utilização futura das amostras biológicas armazenadas no estudo em tela (Item 2.I, da Resolução CNS nº 441 de 2011), se pertinente.


ANÁLISE: PENDÊNCIA ATENDIDA.
3.2 - Declaração de que toda nova pesquisa a ser realizada com o material armazenado em biorrepositório será submetida para aprovação do Comitê de Ética em Pesquisa (CEP) institucional e, quando for o caso, da Comissão Nacional de Ética em Pesquisa (CONEP) (Item 2.III, da Resolução CNS nº 441 de 2011), se pertinente.

RESPOSTA: Declaração anexada (Arquivo: declaração estudos futuros).
ANÁLISE: PENDÊNCIA ATENDIDA.

3.3 - Regulamento dos laboratórios envolvidos no armazenamento do material biológico: detalhamento operacional e de infraestrutura, bem como as condições de armazenamento do material, que podem estar contidos no projeto de pesquisa detalhado ou em forma de declaração. Cabe ressaltar que o prazo de armazenamento de material biológico humano em biorrepositório deve estar de acordo com o cronograma da pesquisa correspondente e pode ser autorizado por até dez anos. Solicita-se explicitar no projeto de pesquisa o tempo de armazenamento.

RESPOSTA: Declarações anexadas (Arquivos: Detalhamento operacional e de infraestrutura do Lanucli e Detalhamento operacional e de infraestrutura do laboratório da Universidade de Surrey) e tempo de armazenamento explicitado no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, item “5.3 AVALIAÇÃO LABORATORIAL” página 18.
ANÁLISE: PENDÊNCIA ATENDIDA.

3.4 - Termo de Acordo em pesquisa envolvendo mais de uma instituição (acordo interinstitucional), assinado, contemplando formas de operacionalização, compartilhamento e utilização do material biológico humano armazenado no Biorrepositório, inclusive a possibilidade de dissolução futura da parceria e a consequente partilha e destinação dos dados e materiais armazenados. Em se tratando de biorrepositório compartilhado, o Termo de Acordo deve ser assinado pelos pesquisadores responsáveis de cada instituição envolvida e pelos seus responsáveis institucionais.

RESPOSTA: Termo de Acordo interinstitucional em pesquisa anexado.
ANÁLISE: PENDÊNCIA ATENDIDA.

3.5 - Declaração garantindo aos pesquisadores e às instituições brasileiras o direito ao acesso e utilização do material biológico humano armazenado no exterior (Resolução CNS 441/2011, item 14).
RESPOSTA: Declaração anexada (Arquivo: Declaração de uso do material biológico no exterior).
ANÁLISE: PENDÊNCIA ATENDIDA.

3.6 - Compromisso dos pesquisadores no exterior quanto à vedação do patenteamento e da utilização comercial do material biológico humano armazenado em biorrepositório (Item 16, da Resolução CNS nº 441 de 2011).
RESPOSTA: Declaração anexada (Arquivo: Declaração de uso do material biológico no exterior).
ANÁLISE: PENDÊNCIA ATENDIDA.

3.7 - Em relação ao envio de material biológico ao exterior, o pesquisador e a instituição nacionais devem estar atentos às normas e disposições legais sobre remessa de material para o exterior e às que protegem a propriedade industrial e/ou transferência tecnológica (Lei nº 9.279 de 14/05/96 que regula direitos e obrigações relativos à propriedade industrial, Decreto nº 2.553/98 que a regulamenta e Lei nº 9.610/98 sobre direito autoral).
RESPOSTA: As pesquisadoras responsáveis por este projeto confirmam estarem atentas às normas e disposições legais sobre remessa de material para o exterior e às que protegem a propriedade industrial e/ou transferência tecnológica.
ANÁLISE: PENDÊNCIA ATENDIDA.

3.8 - No cadastro do Protocolo de Pesquisa na Plataforma Brasil ("PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_781859.pdf" de 18/11/2016) foi informado que não haverá retenção de amostras para armazenamento em banco. O termo "banco" é equivocadamente interpretado como "biobanco", quando, na realidade, aplica-se tanto a biobanco quanto a biorrepositório. Assim, sempre que houver coleta de material biológico em uma pesquisa, este campo da Plataforma Brasil deverá ser assinalado com a opção “SIM”. Solicita-se adequação.
RESPOSTA: Informação corrigida.
ANÁLISE: PENDÊNCIA ATENDIDA.

4 - Quanto ao Termo de Consentimento Livre e Esclarecido:

4.1. Considerando os trechos abaixo:

i. Na página 1 de 8, lê-se: "Os dados coletados nesta pesquisa serão analisados nos laboratórios

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da Universidade de Surrey E OS RESULTADOS SERÃO DISPONIBILIZADOS TAMBÉM À UNIVERSIDADE FEDERAL DE GOIÁS." (Destaque nosso).

ii. Na página 4 de 8, item "DIVULGAÇÃO DOS RESULTADOS", lê-se: Os resultados desta pesquisa serão divulgados das seguintes maneiras: - Consulta para retorno dos resultados dos exames de sangue e da avaliação nutricional aos pacientes que solicitarem este serviço. - Publicação em artigos científicos de revistas internacionais e nacionais; - Dissertação de doutorado.

iii. Na página 6 de 8, lê-se: "4- Todos os resultados obtidos serão confidenciais, sigilosos e privativos. Também é garantido o acesso irrestrito dos voluntários aos resultados do estudo, tendo eles a opção de tomar ou não conhecimento dessas informações."

iv. Na página 5 de 8, no item "GARANTIA DE ESCLARECIMENTO, RECUSA E SIGILO" lê-se: "Os dados serão divulgados de forma anônima e apenas para pesquisa, artigos e eventos científicos, RESPEITANDO A DECLARAÇÃO INTERNACIONAL SOBRE OS DADOS GENÉTICOS HUMANOS. Diante do exposto, o TCLE deve ser explícito em relação à confidencialidade e anonimização dos dados, assegurando que:

4.1.1. Solicita-se mencionar que os dados do participante da pesquisa são confidenciais e somente serão encaminhados a terceiros, como por exemplo, Universidade de Surrey e Universidade Federal de Goiás, após a devida anonimização.


ANÁLISE: PENDÊNCIA ATENDIDA.

4.1.2. Deve-se explicar como será o mecanismo utilizado para garantir a confidencialidade e a anonimização dos dados (exemplo: codificação dos dados, senha de acesso aos bancos de dados, etc.).

RESPOSTA: Texto foi adequado conforme solicitação. Ver documento “Termo de Consentimento Livre e Esclarecido revisado" no item “"GARANTIA DE ESCLARECIMENTO, RECUSA E SIGILO ", página 6, bem como no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE
CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 6.
ANÁLISE: PENDÊNCIA ATENDIDA.

4.1.3. Se o prontuário médico for consultado pelos pesquisadores, esta informação deverá constar no TCLE.
RESPOSTA: Não haverá consulta de prontuário médico pelos pesquisadores.
ANÁLISE: PENDÊNCIA ATENDIDA.

4.1.4. Solicita-se alteração do trecho “RESPEITANDO A DECLARAÇÃO INTERNACIONAL SOBRE OS DADOS GENÉTICOS HUMANOS” para “respeitando as normativas éticas brasileiras homologadas pelo Conselho Nacional de Saúde/Ministério da Saúde do Brasil.”.
ANÁLISE: PENDÊNCIA ATENDIDA.

4.2. Na página 1 de 8, lê-se: “Em caso de dúvida sobre a pesquisa, você poderá entrar em contato a cobrar com os pesquisadores responsáveis, Patrícia Borges Botelho e Marcela Moraes Mendes (62) 3209-6270 (ramal 205), Endereço: Rua 227 Qd. 68 s/nº - Setor Leste Universitário - Goiânia - Goiás - Brasil - CEP: 74.605-08 ou pelo endereço de email dsol@surrey.ac.uk.”. Solicita-se informar ainda um meio de contato de fácil acesso ao participante de pesquisa em caso de urgência (24 horas por dia, 7 dias por semana).
ANÁLISE: PENDÊNCIA ATENDIDA.

4.3. Na página 1 de 8, lê-se: “Ela foi criada pela Resolução do CNS 196/96 e tem como principal função examinar os aspectos éticos das pesquisas que envolvem seres humanos.”. Solicita-se exclusão desse trecho para evitar confundir o participante de pesquisa, visto que a Resolução CNS 196/96 já foi revogada.
RESPOSTA: Trecho excluído como solicitado.
ANÁLISE: PENDÊNCIA ATENDIDA.

4.4. Na página 1 de 8, lê-se: "A CONEP está localizada na Esplanada dos Ministérios (Ministério da Saúde) no Edifício Anexo Bloco G Ala B Sala 13-B, e em caso de qualquer dúvida sobre seus direitos como participante da pesquisa você poderá entrar em contato com a CONEP pelo telefone (61) 3315-2951 no horário de 08 às 17h de segunda a sexta ou pelo e-mail conep@saude.gov.br.". Solicita-se alterar o trecho para "A CONEP está localizada no Setor de Edifícios Públicos Norte - SEPN 510 NORTE, BLOCO A, 3º Andar Edifício Ex-INAN - Unidade II - Ministério da Saúde; CEP: 70750-521 - Brasília-DF, e em caso de qualquer dúvida sobre seus direitos como participante da pesquisa você poderá entrar em contato com a CONEP pelo telefone (61) 3315-5881 no horário de 08 às 20h de segunda a sexta ou pelo e-mail conep@saude.gov.br."

RESPOSTA: Texto foi alterado conforme solicitação. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” página 1, bem como no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 1.

ANÁLISE: PENDÊNCIA ATENDIDA.

4.5. Na página 2 de 8, lê-se: "A deficiência de vitamina D é considerada um importante problema de saúde pública por suas implicações em diversas doenças como osteoporose, doenças cardíacas, diabetes tipo I e II, DOENÇAS INFLAMATÓRIAS E AUTO-IMUNES e esclerose múltipla." (Destaque nosso). Solicita-se exemplificar doenças inflamatórias e autoimunes.


ANÁLISE: PENDÊNCIA ATENDIDA.

4.6. Na página 2 de 8, solicita-se incluir informações acerca da dose diária de suplementação de vitamina D (600IU) por 12 semanas.


ANÁLISE: PENDÊNCIA ATENDIDA.
4.7. Solicita-se esclarecer sobre a possibilidade de inclusão do participante em grupo controle ou placebo, explicitando, claramente, o significado dessa possibilidade.


ANÁLISE: PENDÊNCIA ATENDIDA.

4.8. Na página 2 de 8, lê-se: “Todos os resultados que conseguirmos com os seus dados e amostras estarão disponíveis para você, caso queira recebê-los ou não, e eles não serão divulgados sem a sua autorização. VOCÊ PODERÁ SOLICITAR UMA CONSULTA RETORNO PARA RECEBER OS RESULTADOS DOS SEUS EXAMES A PARTIR DE DEZEMBRO DE 2017.”. Solicita-se adequação do trecho, tendo em vista que, caso o participante necessite de orientação, deve ser agendada a qualquer momento que ele necessite.


ANÁLISE: PENDÊNCIA ATENDIDA.

4.9. Na página 3 de 8, item “RESSARCIMENTO”, lê-se: “Você não terá nenhum gasto para participar da pesquisa e será ressarcido (você e acompanhantes) das despesas que a pesquisa possa oferecer, NA FORMA DE BILHETES OU O DINHEIRO DA (S) PASSAGEM (NS) DO TRANSPORTE PÚBLICO PARA O DESLOCAMENTO PARA AS ATIVIDADES DA PESQUISA.” (Destaque nosso). O ressarcimento é a compensação material, exclusivamente de despesas do participante e seus acompanhantes, quando necessário, tais como transporte (mas não limitado a transporte público ou bilhetes) e alimentação (II.21. da Resolução CNS nº 466 de 2012). Portanto, o TCLE deve assegurar de forma clara e afirmativa o ressarcimento de todos os gastos que o participante e seu(s) acompanhante(s) terão ao participar da pesquisa.

RESPOSTA: O texto foi alterado para assegurar de forma clara e afirmativa o ressarcimento de todos os gastos que o participante possa ter com a pesquisa, conforme solicitação. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” item “RESSARCIMENTO” página 5, bem como no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 5.
ANÁLISE: PENDÊNCIA ATENDIDA.

4.10. Na página 3 de 8, item “RESSARCIMENTO”, lê-se: “A PESQUISA SERÁ IMEDIATAMENTE INTERROMPIDA SE OFERECER ALGUM RISCO OU DANO À SUA SAÚDE” (Destaque nosso). Ainda, na página 7 de 8, lê-se: “8- A pesquisa será imediatamente interrompida se for percebido algum risco ou dano à saúde do participante da pesquisa, consequente à mesma, não previsto no termo de consentimento.”. Cabe lembrar que, de acordo com o item V.3 da Resolução CNS Nº 466 de 2012, “O pesquisador responsável, ao perceber qualquer risco ou dano significativos ao participante da pesquisa, previstos, ou não, no Termo de Consentimento Livre e Esclarecido, DEVE COMUNICAR O FATO, IMEDIATAMENTE, AO SISTEMA CEP/CONEP, e avaliar, em caráter emergencial, a necessidade de adequar ou suspender o estudo.”. Solicita-se adequação.

RESPONSA: Documento foi adequado de forma a incluir trecho solicitado. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” item “ESCLARECIMENTOS DADOS PELO PESQUISADOR SOBRE A GARANTIA DO SUJEITO DA PESQUISA”, número 9, página 8, bem como no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 8.

ANÁLISE: PENDÊNCIA ATENDIDA.

4.11. Na página 3 de 8, item “RESSARCIMENTO”, lê-se: “e caso ocorra algum prejuízo causado pela participação na pesquisa, você receberá A ASSISTÊNCIA, ACOMPANHAMENTO e indenização, sob responsabilidade dos pesquisadores e da Faculdade de Nutrição da Universidade Federal de Goiás.” (Destaque nosso). Ainda na página 7 de 8, lê-se: “8- A pesquisa será imediatamente interrompida se for percebido algum risco ou dano à saúde do participante da pesquisa, consequente à mesma, não previsto no termo de consentimento.”. O TCLE deve assegurar, de forma clara e afirmativa, que o participante da pesquisa receberá a assistência integral e imediata, de forma gratuita, pelo tempo que for necessário em caso de danos decorrentes da pesquisa.

RESPONSA: O documento foi adequado para assegurar, de forma clara e afirmativa, que o participante da pesquisa receberá a assistência integral e imediata, de forma gratuita, pelo tempo que for necessário em caso de danos decorrentes da pesquisa. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” item “ESCLARECIMENTOS DADOS PELO PESQUISADOR SOBRE A GARANTIA DO SUJEITO DA PESQUISA”, número 8, página 8, bem como no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 8.
ANÁLISE: PENDÊNCIA ATENDIDA.

4.12. Solicita-se correção do número das páginas, tendo em vista que a numeração se encontra entre alguns trechos do TCLE. Solicitam-se adequações para que a numeração possa ser encontrada no início ou no fim de cada página.
RESPONSA: A paginação do documento TCLE foi formatada.
ANÁLISE: PENDÊNCIA ATENDIDA.

4.13. Na página 5 de 8, lê-se: "Não foram identificados riscos relacionados a ingestão de cápsulas de vitamina D em pesquisas anteriores." Sabe-se que o excesso de vitamina D pode ter efeitos secundários. Pode ter efeitos colaterais nos rins, no coração e sistema cardiovascular, como arritmia cardíaca e sopro cardíaco. Outros sintomas de excesso de vitamina D incluem náuseas, perda de peso etc. Consumir muita vitamina D durante a gravidez pode aumentar o nível de cálcio no sangue que podem levar à deficiência mental e deficiência física como malformação da estrutura óssea do feto. A ingestão excessiva de vitamina D também pode causar alguns defeitos congênitos. Portanto, os riscos do estudo com relação à ingestão de vitamina D não devem ser subestimados. Solicita-se adequação informando os potenciais riscos associados ao uso da vitamina D e/ou outros riscos também associados à pesquisa explicitando ainda as providências e as cautelas a serem tomadas para evitar que ocorram os riscos.
RESPONSA: O texto foi adequado conforme solicitação, explicitando os riscos e a forma como serão minimizados. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” no item “DESCONFORTOS E RISCOS ESPERADOS página 5 e 6, bem como no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 5 e 6.
ANÁLISE: PENDÊNCIA ATENDIDA.

4.14. Na página 5 de 8, item “BENEFÍCIOS QUE PODERÃO SER OBTIDOS” lê-se: "VOCÊ TERÁ ACESSO AOS RESULTADOS DOS SEUS EXAMES (EXAME DE SANGUE, SAÚDE DO OSSO E PORCENTAGEM DE GORDURA CORPORAL, E EXAME GENÉTICO) GRATUITAMENTE POR MEIO DE CONSULTA RETORNO. Os resultados gerados desta pesquisa irão contribuir para sejam elaboradas melhores recomendações e orientações de saúde em relação à vitamina D e mais específicas e eficazes para mulheres brasileiras." O TCLE deve apresentar, de forma clara e objetiva, os potenciais benefícios da pesquisa ao participante, sem supervalorizá-los. Portanto, solicita-se exclusão do trecho "VOCÊ
TERÁ ACESSO AOS RESULTADOS DOS SEUS EXAMES (EXAME DE SANGUE, SAÚDE DO OSSO E PORCENTAGEM DE GORDURA CORPORAL, E EXAME GENÉTICO) GRATUITAMENTE POR MEIO DE CONSULTA RETORNO* por se tratar de um direito do participante de pesquisa (e não benefício). Caso o estudo não antecipe qualquer benefício direto ao participante, essa informação deve constar do TCLE de forma explícita. Solicita-se adequação.

**RESPOSTA:** O texto foi adequado conforme solicitação. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” no item “BENEFÍCIOS QUE PODERÃO SER OBTIDOS” página 6, bem como no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 6.

**ANÁLISE:** PENDÊNCIA ATENDIDA.


**RESPOSTA:** Alterado conforme solicitado. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” no item “ESCLARECIMENTOS DADOS PELO PESQUISADOR SOBRE A GARANTIA DO PARTICIPANTES DA PESQUISA” página 7, bem como no projeto detalhado “2_DSOL_PROTOCOLO REVISADO.pdf”, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 7.

**ANÁLISE:** PENDÊNCIA ATENDIDA.

4.16. Considerando que haverá análise genética, e em cumprimento da Resolução CNS nº 340 de 2004, solicita-se adequação quanto às seguintes informações:

4.16.1. O TCLE deve trazer, de forma explícita, os genes/segmentos de DNA/RNA que serão estudados. Contudo, se for inviável do ponto de vista prático listar todos os genes, é aceitável que o pesquisador descreva os genes a serem estudados de forma agrupada segundo funcionalidade ou efeito (carta circular CONEP 041/2015).

**RESPOSTA:** Os genes/segmentos de DNA/RNA que serão estudados foram explicitados como solicitado. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” no item “ANÁLISE GENÉTICA” página 4, bem como no projeto detalhado 2_DSOL_PROTOCOLO REVISADO, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 4.

**ANÁLISE:** PENDÊNCIA ATENDIDA.

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**Endereço:** SEPN 510 NORTE, BLOCO A 3º ANDAR, Edifício Ex-INAN - Unidade II - Ministério da Saúde  
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**Telefone:** (61)3315-5878  
**E-mail:** conep@saude.gov.br
4.16.2. O TCLE deve assegurar, de forma clara e afirmativa, que os dados genéticos são confidenciais e que não serão repassados a terceiros (como, por exemplo: seguradoras, empregadores, supervisores hierárquicos, entre outros). Além do mais, os mecanismos de proteção dos dados genéticos devem ser explicados no TCLE.

**RESPOSTA:** O texto foi adequado, conforme solicitação. Ver documento “Termo de Consentimento Livre e Esclarecido revisado” no item “GARANTIA DE ESCLARECIMENTO, RECUSA E SIGILO ”, página 6, bem como no projeto detalhado 2_DSOL_PROTOCOLO REVISADO, APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 6.

**ANÁLISE:** PENDÊNCIA ATENDIDA.

4.16.3. Quando aplicável, o TCLE deve assegurar, de forma clara e afirmativa, que o patrocinador oferecerá ao participante de pesquisa o aconselhamento genético e o acompanhamento clínico necessários. Deve-se informar, também, quem realizará esses procedimentos (ou onde serão realizados).

**RESPOSTA:** Não aplicável.

**ANÁLISE:** PENDÊNCIA ATENDIDA.

4.16.4. O TCLE deve assegurar, de forma clara e afirmativa, que os resultados de exames serão informados ao participante de pesquisa se assim ele quiser. O texto foi adequado conforme solicitação.

**RESPOSTA:** Ver documento “Termo de Consentimento Livre e Esclarecido revisado” no item “ANÁLISE GENÉTICA” página 4 e “DIVULGAÇÃO DOS RESULTADOS “, na página 5, bem como no projeto detalhado 2_DSOL_PROTOCOLO REVISADO , APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE), página 4 e 5.

**ANÁLISE:** PENDÊNCIA ATENDIDA.

4.16.5. Quando aplicável, o TCLE deve informar que o resultado dos exames genéticos pode trazer riscos ao participante de pesquisa. Neste caso, o TCLE deve informar que o participante tem a opção de conhecer ou não o resultado desses exames.

**RESPOSTA:** Não aplicável.

**ANÁLISE:** PENDÊNCIA ATENDIDA.

4.17. No TCLE não há menção do destino das amostras biológicas ao final do estudo. Cabe
ressaltar que o prazo de armazenamento de material biológico humano em biorrepositório deve estar de acordo com o cronograma da pesquisa correspondente e pode ser autorizado por até dez anos. O TCLE deve conter o consentimento de autorização para a coleta, armazenamento e utilização das amostras biológicas conforme a Resolução CNS 441/11 e Portaria MS 2.201/11. O documento deve informar se as amostras serão utilizadas apenas para os propósitos descritos no protocolo (e destruídas após a sua utilização) e/ou se haverá armazenamento para utilização em investigações futuras. Solicita-se adequar o documento, esclarecendo ao participante que, em caso de utilização das amostras em pesquisas futuras, ele será contatado novamente para fins de convite e autorização expressa do novo uso.

RESPOSTA: Informação adicionada no item “ARMAZENAMENTO DE MATERIAL BIOLÓGICO, página 7 do documento “Termo de Consentimento Livre e Esclarecido revisado” e do projeto detalhado 2_DSOL_PROTOCOLO REVISADO , APÊNDICE 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE).

ANÁLISE: PENDÊNCIA ATENDIDA.

4.18. O documento apresenta inúmeros erros de digitação que podem interferir na autonomia do participante da pesquisa. Solicita-se adequação.

RESPOSTA: O documento foi revisado por completo para correção de erros de digitação.

ANÁLISE: PENDÊNCIA ATENDIDA.

4.19. O documento apresenta diversos termos técnicos que podem não ser de compreensão do participante da pesquisa, interferindo em sua autonomia no momento de decisão. Solicita-se a retirada de tais termos, ou que após a citação haja uma breve explicação de seu significado.

RESPOSTA: O documento foi revisado por completo para substituição/explicação de termos técnicos.

ANÁLISE: PENDÊNCIA ATENDIDA.

4.20. Solicitam-se esclarecimentos e/ou adequação em relação aos trechos “... na qual serão avaliadas moléculas presentes na sua SALIVA” e “... durante a COLETA DE SALIVA ou na entrevista...” (destaques nosso), considerando que o projeto não prevê a coleta de saliva.

RESPOSTA: O termo utilizado está inadequado e foi substituído para “sangue”.

ANÁLISE: PENDÊNCIA ATENDIDA.
4.21. Na página 6 de 8, lê-se “Autorizo armazenagem armazenamento e a utilização do material biológico (amostras de sangue) até o final desta pesquisa: ( ) Sim ( ) Não”. Solicita-se exclusão do trecho nesta parte do documento, pois não corresponde à sequência das informações apresentadas.

RESPOSTA: O termo foi retirado, como solicitado.

ANÁLISE: PENDÊNCIA ATENDIDA.

4.22. Considerando que o estudo prevê apenas o recrutamento de participantes femininas, solicita-se adequação de trechos, tais como “Você está sendo convidado (a) “, “voluntário (a) “, “você não será penalizado (a) “, “Vocé será informado”, etc.

RESPOSTA: Os trechos foram corrigidos, conforme solicitado.

ANÁLISE: PENDÊNCIA ATENDIDA.

4.23. Todos os aspectos abordados no TCLE foram destinados a mulheres adultas brasileiras residentes no Brasil, por exemplo: “Entrevista que ocorrerá na Clínica escola da FANUTUFG”, “Após 3 meses, você deverá retornar a Clínica Escola...”, “...você receberá a assistência, acompanhamento e indenização, sob responsabilidade dos pesquisadores e da Faculdade de Nutrição da Universidade Federal de Goiás”, “As suas amostras de sangue serão guardadas na Faculdade de Nutricao, Universidade Federal de Goiás...”. Considerando que a primeira fase do projeto será executada no Reino Unido, solicita-se adequação do documento contemplando também a realidade das participantes brasileiras residentes no exterior.

RESPOSTA: A primeira fase do projeto, que inclui apenas mulheres brasileiras residentes no Reino Unido, já ocorreu e estas foram contempladas nos documentos aprovados pelo Comitê de Ética da Universidade de Surrey, incluindo TCLE específico para esta fase do projeto. Pedimos cordialmente reconsideração desta solicitação, por entendermos que neste TCLE não é necessário mencionar tal informação, pois refletiria uma realidade das participantes residentes no Reino Unido e não das do Brasil, o que poderia acabar por confundir as participantes as quais este TCLE se aplica. Acréscimo de informação no Protocolo de Pesquisa Aproveitamos a oportunidade, para comunicar o acréscimo de informação a respeito de uma análise adicional de expressão gênica de RNAms no projeto detalhado 2_DSOL_PROTOCOLO REVISADO nos itens “2.5 INFLUÊNCIA DOS FATORES GENÉTICOS SOBRE A CONCENTRAÇÃO DE VITAMINA D” página 9 e 10, e “5.8 EXTRAÇÃO DE DNA E DETERMINAÇÃO DOS POLIMORFISMSOS E EXPRESSÃO GÊNICA” página 21.

ANÁLISE: PENDÊNCIA ATENDIDA.
Considerações Finais a critério da CONEP:
Diante do exposto, a Comissão Nacional de Ética em Pesquisa - Conep, de acordo com as atribuições definidas na Resolução CNS nº 466 de 2012 e na Norma Operacional nº 001 de 2013 do CNS, manifesta-se pela aprovação do projeto de pesquisa proposto, devendo o CEP verificar o cumprimento das questões acima, antes do início do estudo.

Situação: Protocolo aprovado com recomendação.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

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### COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

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### Situação do Parecer:
Aprovado com Recomendação

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**CEP:** 70.750-521
**E-mail:** cone@saude.gov.br

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BRASILIA, 21 de Março de 2017

Assinado por:
Jorge Alves de Almeida Venancio
(Coordenador)
THE D-SOL STUDY

Vitamin D supplementation and sunlight exposure in Brazilian women living in opposite latitudes

Consent Form

Please initial each box

• I have read and understood the Information Sheet provided (version 8, date 14/11/16). I have been given a full explanation by the investigators of the nature, purpose, location and likely duration of the study, and of what I will be expected to do.

• I have been advised about any disadvantages/discomfort which may result from participating. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result.

• I agree to comply with the requirements of the study as outlined to me to the best of my abilities.

• I understand that in accordance with the English law, insurance is in place which covers harm that is likely to result from my participation in this study as detailed in the participant information sheet.

• I agree for my anonymised data and/or samples to be used for this study that will have received all relevant legal, professional and ethical approvals.

• I agree to be part in the genetic study to be conducted after the trial, as detailed in the participant information sheet, and allow my data and blood sample to be used for genetic analyses.

• I understand that all project data will be held for at least 6 years and all research data for at least 10 years in accordance with University policy and that my personal data is held and processed in the strictest confidence, and in accordance with the UK Data Protection Act (1998).

• I agree for the researchers to contact me about future studies.

D-SOL Consent Form
Version 5.0 30/08/2016

When completed: 1 for participant; 1 for researcher site file.
• I agree for my personal and research data to be transferred to Federal University of Goias – Brazil. I understand that my personal data may not be held under the same stringent laws as in the UK.

• If I withdraw from the study, unless I specifically request my data be withdrawn, it will still be used in the analysis.

• I understand that I can request withdraw of my data until the end of data collection (March 2017), without needing to justify my decision, without prejudice and without my legal rights being affected.

• I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation.

Name of participant (BLOCK CAPITALS) .............................................................

Signed ...........................................................................................

Date ..............................................................................................

Name of researcher taking consent (BLOCK CAPITALS) .............................................................

Signed ...........................................................................................

Date ..............................................................................................
Vitamin D and Sunlight exposure in Brazilian women living in Opposite Latitudes

The D-SOL study wants to investigate which factors may influence the response to vitamin D supplementation in people living in opposite latitudes.

If you are a BRAZILIAN WOMAN living in the UK, aged 20 – 59 years,

we would like to hear from you!

You will be asked to visit us on 2 occasions at the University of Surrey and must be willing to take vitamin D supplement tablets every day during the study (3 months).

Any travel costs will be reimbursed!

For more information please contact: Marcela Mendes (PhD Research Fellow) on +44 (0) 1483 689222 or dsol@surrey.ac.uk

Recruitment end date: 16th November 2016

This study has been reviewed by and received a favourable ethical opinion from the University of Surrey Ethics Committee.
Invitation Letter to the D-SOL Study

10/09/2016

Dear Ms./Mrs.,

The Nutritional Sciences research team at the University of Surrey is writing to inform you of a new study called “Vitamin D Supplementation and sunlight exposure in Brazilian women living in Opposite Latitudes” (the D-SOL Study), that will take place over the next year, in collaboration with the Federal University of Goias, Brazil. This research is funded by the Science Without Borders Program.

Our research team is interested in looking at the importance of vitamin D supplementation and sunlight exposure to the maintenance of adequate vitamin D levels, and the influence of diet, skin colouring and genetics for optimal vitamin D status, in Brazilian women living in Brazil and in the UK. Several studies from sunny countries have shown that vitamin D deficiency is a common problem, despite the abundance of sunlight in these locations making it a global health problem, emphasizing the importance to study vitamin D in both countries.

To take part in this study, you would be asked to visit us on two separate dates, 3 months apart (visits can be made at a day which is convenient for you). Your participation would involve completing a range of brief questionnaires, using a small badge on your clothes for one week to measure sunlight exposure, taking some body measures (height, weight and waist circumference), a full-body bone scan and blood sample collection. You would also be asked to take vitamin D supplement tablets daily (which may contain vitamin D or will be a placebo containing no vitamin D) for a period of 3 months.

Upon completion of the study, you can request information on your bone health and nutritional status, as well as vitamin D levels. Any travel costs will be reimbursed.

If you are interested in learning more about this study, please contact us at dsol@surrey.ac.uk or +44 (0) 1483 689222.

We appreciate your time and consideration and look forward to hearing from you.

Sincerely on behalf of D-SOL Team,

Marcela M. Mendes (PhD Research Fellow)

Professor Susan Lanham-New (Academic Supervisor)
THE D-SOL STUDY

Participant Information Sheet
Version 6, 13/09/2016

Vitamin D supplementation and sunlight exposure in Brazilian women living in opposite latitudes
(The D-SOL Study)

Introduction

We, the D-SOL team from the University of Surrey, would like to invite you to take part in a research project as part of a PhD program, funded by the Science without Borders. Before you decide whether you would like to participate, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and ask questions about anything you do not understand. Talk to others about the study if you wish.

Please do not hesitate to ask us if there is anything that is not clear or if you would like any more information (contact Miss Marcela Mendes, PhD Research Fellow and Principal Investigator of this study on email dsol@surrey.ac.uk or telephone +44 (0) 1483 689222)

What is the purpose of the study?

It is generally believed that the major source of vitamin D is the exposure of the skin to UV B-rays contained in sunlight. However, several studies from sunny countries have shown that vitamin D deficiency is common, despite the abundance of sunlight in these locations. We still don’t know how much of our vitamin D comes from our food and how much comes from sun in different countries including the UK and Brazil. Also we know that not everyone gets the same benefit from supplements and this may be due to genetic differences.

Therefore we want to look at the importance of vitamin D supplementation and sunlight exposure to the maintenance of adequate vitamin D levels, and the influence of diet, skin coloring and genetics for optimal vitamin D status, comparing Brazilian women living in Brazil, where there is abundant sunlight exposure and Brazilian women living in the UK, where there is limited exposure to adequate sunlight.
Why have I been invited to take part in the study?

You have been invited to participate on our study as you meet the following criteria: Brazilian, aged 20-59 years and female.

To be eligible to take part in the study, you must meet the following criteria:

- Brazilian
- Female
- Aged 20 – 59 years
- Not currently suffering from any conditions or taking any medication likely to affect your vitamin D status and bone metabolism as listed in the screening questionnaire (exclusion criteria).

About 80 participants living in Brazil will take part in this study and 80 participants living in the UK.

Do I have to take part?

No, you do not have to participate. You can withdraw at any time without giving a reason and without prejudice. Data collection will take place from November 2016 to March 2017.

If you withdraw from the study this will mean the following for your participation and data: with your permission, we intend on retaining data already collected. However, no further data will be collected nor will any other research procedure be carried out on you. If you wish, you can contact us to request the data be withdrawn up to the end of data collection (March 2017). Unless you specifically request the data to be withdrawn it will still be used in the analysis.

What will my involvement require?

If you are interested in taking part on our study, you will be asked to answer a screening questionnaire, either by email or phone, and receive this information sheet by post or email.

If you are eligible for the study and wish to participate you will be invited to come to your first visit. On this visit, you will be given the opportunity to clarify any doubts you may have regarding the study. If you are still happy to take part, you will then be asked to sign a consent form and will receive a copy of your consent form and this information sheet, to keep for yourself.
The research will last 1 year but you would only be asked to visit us at the Clinical Investigation Unit at the University of Surrey, Guildford, on two separate dates (3 months apart). Visits can be made at a day which is convenient for you.

On your first visit you will be asked to:
- complete 1 questionnaire;
- go through a dual energy X-ray absorptiometry (DEXA) scan*;
- have your weight, height and waist circumference measured and blood sample (equivalent of 5 teaspoons) collected by a trained phlebotomist;
- have your melanin amount in your upper and forearm skin measured by a reflectance spectrophotometer**;
- complete food and activity diaries for 4 days;
- wear a small dosimeter badge on your clothes for a week;
- take Vitamin D or placebo supplements (provided by the study) daily for the total period of 3 months.

On your second (final) visit you will be asked to:
- have your weight, height and waist circumference measured and blood sample collected;
- have your melanin amount in your upper and forearm skin measured by a reflectance spectrophotometer**;
- return completed food and activity diaries;
- return dosimeter badge;
- return supplements emptied bottles.

* A DEXA (DXA) scan is a quick and painless procedure that involves lying on your back on an X-ray table so that an area of your body can be scanned. No special preparations are needed before having a DEXA scan, during which X-rays will be passed through your body. DEXA scans use a much lower level of radiation than standard X-ray examinations.

** A reflectance spectrophotometer is a very easy and non-invasive quick tool to determine the colour of the skin, where a receiver measures the light reflected by skin pigmentation.
After your final visit you might be select to be part on a genetic analysis to determine the influence of genetic mutation on the vitamin D supplementation response. On your first visit we will ask for your consent to have your blood sample used for this purpose. However, being part on the genetic analysis does not require any further visits from you.

The visits will be held in December 2016 and February/March 2017 on a day and at a time which is suitable for you.

At your first visit you will be given 12 weeks supply of small tablets, which may contain vitamin D or will be a placebo containing no vitamin D.

Throughout the study we will also keep in touch with you by telephone in 5 occasions (2 weeks apart from each other), to see how you are getting on with the study.

Any travel costs will be reimbursed.

After the completion of the study, the D-SOL team may wish to contact you regarding future studies. However, you do not have to participate again if you do not wish to. You can refuse to take part in any future studies without giving a reason and without prejudice.

What will I have to do?

First we will ask you some questions via a phone call to make sure you are eligible to take part in the study. We will then invite you to visit us on two occasions. On your first visit we will go through all the details of the study and discuss any questions you may have. Then you will be asked to sign a consent form, which means you are happy with all the information you have received and you are willing to take part on our study. The visits will take around 30 minutes to complete and will be scheduled in the morning on a day of your choice.

What will happen to data/samples that I provide?

Research data are stored securely for at least 10 years following their last access and project data (related to the administration of the project, e.g. your consent form) for at least 6 years in line with the University of Surrey policies.

Personal data will be handled in accordance with the UK Data Protection Act (1998).

With your consent, to make the most of your participation and support efficient advancements in science, any anonymised data/samples may be used for future research. We cannot tell you at this moment in time what this research will entail or what analyses will be carried
out but we can assure you that all appropriate legal, ethical and other approvals will be in place. For practical reasons your consent will not be sought again.

**What are the possible disadvantages or risks of taking part?**

Study visits might be inconvenient as they required coming to the University during work hours. Some people may also find taking supplement tablets everyday and/or keeping diaries inconvenient or unpleasant. Some may also find taking blood sample unpleasant, and due to the nature of the procedure, some light bruising may occur.

DEXA scans use a low level of radiation (much lower than standard X-ray examination) to which you will be exposed. The amount of radiation absorbed from the scan is very small and similar to the radiation we receive from the environment.

Throughout the duration of the trial, you will be contacted via telephone on a fortnightly basis to discuss any issues with any adverse event and compliance and to maintain good communication with our team.

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

You will receive information on your bone health and nutritional status, as well as vitamin D levels. The information that you provide to this study will be of great value to science and will provide both countries with key data on whether there should be consideration of further revisions to dietary recommendations for vitamin D in adult populations. Even more, contributing to a study that focus on Brazilian women, you will be helping to determine specific considerations regarding vitamin D for this group, in which you are included and therefore directly benefited.

**What happens when the research study stops?**

Once the study finishes, the data collected will begin to be analysed. As part of the collaboration agreement with the Federal University of Goias, Brazil, all data collected in both countries will be shared between the two institutions. This means that personal and research data will be transferred to Federal University of Goias – Brazil and may not be held under the same stringent laws as in the UK.
Once we are done with all the data collection and examination, if you wish, you can request a report with our overall findings and your personal results, from October 2017.

**What if there is a problem?**

If you are unsatisfied with any aspect of the study please contact Miss Marcela Mendes (email: m.moraesmendes@surrey.ac.uk or telephone 01483 689222) or Prof. Susan Lanham-New, PhD Supervisor (email: s.lanham-new@surrey.ac.uk or telephone 01483 686476) or Prof. Bruce Griffins, Professor of Nutritional Metabolism at the University of Surrey (email: b.griffin@surrey.ac.uk or telephone 01483 68 9724).

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

Yes. Your details will be held in complete confidence and we will follow ethical and legal practice in relation to all study procedures, including data shared with the collaborative institution, although this shared data may not be held under the same stringent laws as in the UK. For thesis and publications purposes, you will be identified by a unique code and not your name.

**Full contact details of researcher and academic supervisor**

Name: Miss Marcela Moraes Mendes (PhD research fellow)  
Address: Department of Nutritional Sciences, Faculty of Health & Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH  
Telephone: +44 (0) 1483 689222  
Email: m.moraesmendes@surrey.ac.uk

Name: Professor Susan Lanham-New (Academic supervisor)  
Address: Department of Nutritional Sciences, Faculty of Health & Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH  
Telephone: +44 (0) 1483 6896476  
Email: s.lanham-new@surrey.ac.uk
Who is organising and funding the research?

This research is organised by the University of Surrey, UK, and by the Federal University of Goias, Brazil, and funded by the Science Without Borders Program.

Who has reviewed the project?

This study has been reviewed by and received a favorable ethical opinion from the University of Surrey Ethics Committee.

Thank you for taking the time to read this Information Sheet.
SECTION A

THE D-SOL STUDY SCREENING QUESTIONNAIRE

The purpose of this questionnaire is to assess whether you are suitable to take part in the study and that it is safe for you to do so. We would be grateful if you would answer the following questions, even if you are still not sure if you wish to take part in our study. Please answer the questions as honestly and accurately as you can and remember there are no right or wrong answers to the questions. Your answers to the questions on this questionnaire will also be kept completely confidential. If you feel uncomfortable answering any of the questions on this questionnaire you do not have to answer the question. Please ask one of the D-SOL Research Team if you would like help answering any of the questions.

CONTACT DETAILS

Full name: Click here to enter text.
Date of birth: Click here to enter text.
Address: Click here to enter text.
City: Click here to enter text.
County: Click here to enter text.
Contact telephone number: Click here to enter text.
Place of birth: Click here to enter text.
Nationality: Click here to enter text.
Mother’s nationality: Click here to enter text.
Father’s nationality: Click here to enter text.
Email address: Click here to enter text.
Preferred method of contact: ☐ Phone ☐ Email ☐ Post

HEALTH AND LIFESTYLE

Height (cm): Click here to enter text. Weight (kg): Click here to enter text.

Are you currently receiving treatment for any medical conditions? ☐ Yes ☐ No

Medical condition: Click here to enter text. Treatment: Click here to enter text.

Are you on any medication prescribed by your GP or any other health care provider? ☐ Yes ☐ No

If yes, please specify: Click here to enter text.
1. Do you live in the UK? ☐ Yes ☐ No
   If yes, please state the date you arrived:  [Click here to enter text.]

2. Please tick all medical conditions that apply to you:
   - ☐ Prior/present history of coronary heart disease, angina, heart attack or stroke
   - ☐ Prior/present history of Type 1 and Type 2 Diabetes.
   - ☐ Prior/present history of Thyroid disease
   - ☐ Prior/present history of osteoporosis, osteopenia or other musculoskeletal disease
   - ☐ Prior/present history of haematological disease (except mild anaemia)
   - ☐ Prior/present history of malignancy
   - ☐ Prior/present history of a gastrointestinal disorder, such as Crohns Disease, Coeliac Disease or Irritable Bowel Syndrome.
   - ☐ Prior/present history of liver or kidney disease.

3. Do you have any allergies or food intolerances? ☐ Yes ☐ No
   If yes, please specify:  [Click here to enter text.]

4. Do you regularly take vitamin supplements containing vitamin D?
   ☐ Yes ☐ No
   If yes please specify:  [Click here to enter text.]
   How many months you have been taking supplements?  [Click here to enter text.]
   The dose of the supplements (if known):  [Click here to enter text.]
   The brand of the supplements (if known):  [Click here to enter text.]

5. Are you currently on a weight-reducing diet or other dietary restrictions (except vegetarianism)? ☐ Yes ☐ No
   If yes, please provide details:  [Click here to enter text.]

6. Have you been abroad on holiday during the past 6 months? ☐ Yes ☐ No
   If yes, please specify where this was and the month this holiday was taken:
   Country of visit:  [Click here to enter text.]
   Month/year:  [Click here to enter text.]
   Country of visit:  [Click here to enter text.]
   Month/year:  [Click here to enter text.]

6. Are you planning any holidays abroad during the next 12 months? ☐ Yes ☐ No
   If yes, please specify where this visit is planned to be and when this holiday will be taken:
   Country of visit:  [Click here to enter text.]
Month/year: Click here to enter text.
Country of visit: Click here to enter text.
Month/year: Click here to enter text.

7. Do you use sunbeds? ☐ Yes ☐ No
   
   If yes, please state how often you use them:  Choose an item.

   Are you currently pregnant or planning a pregnancy during the next 12 months?
   ☐ Yes ☐ No

8. Are you currently breastfeeding?
   ☐ Yes ☐ No

9. Are you in menopause?
   ☐ Yes ☐ No

We are planning to see participants from in the morning between 8.00am and 12.00 noon in December 2016 and February/March 2017. Are there any particular day or days which would be best for you?

Click here to enter text.

Thank you for taking the time to complete this questionnaire.

Participant signature: Click here to enter text.
Date: Click here to enter text.
THE D-SOL STUDY

Thank you for expressing an interest in participating in this research study. We would be grateful if you would answer the following questions.

Prior to completing this questionnaire please read the Participant Information sheet and complete the consent form if you would like to take part. This questionnaire should take you no more than 15 minutes to complete and we thank you for your time.

PERSONAL AND CONTACT DETAILS

Participant name: ______________________________________________________________
DOB: ___/___/_______
Address: _____________________________________________________________________
Post code: __________
Contact telephone number: __________________________
Nationality: ___________________________________________________________________
Email address: _______________________________________________________________
Preferred method of contact: ( ) Phone ( ) Email ( ) Post
1. Your colour or race is:
(  ) White  (  ) Black  (  ) Brown (Pardo)  (  ) Yellow (Amarelo)  (  ) Indigenous

2. Are you planning to travel abroad frequently between December 2016 and February 2017?
(  ) Yes  (  ) No
If yes, please specify where this visit is planned to be and when this holiday will be taken:
Country of visit: ______________  Month/year: ______________
Country of visit: ______________  Month/year: ______________
Country of visit: ______________  Month/year: ______________

3. In the past three years have you suffered from any of the following?
(  ) Osteoporosis  (  ) Severe Chrons disease  (  ) Asthma
(  ) Coeliac disease  (  ) Breast Cancer  (  ) Skin Cancer
(  ) Thyroid Disease  (  ) Severe Rheumatoid Arthritis
(  ) Any other disease: _______________________________________

4. Do you or have you ever taken any of the following over the last year?
(  ) Hormone Replacement Therapy (HRT)  (  ) Diuretics
(  ) Steroids (tablets or inhales)  (  ) Tamoxifen  (  ) Bone medications
(  ) Any other medication (if yes, please give details): _______________________________

5. Are you or might you be pregnant? (  ) Yes  (  ) No

6. Are you intending to become pregnant in the next year? (  ) Yes  (  ) No

7. Have you had a baby in the last 3 months? (  ) Yes  (  ) No

8. Are you breastfeeding? (  ) Yes  (  ) No
9. How many years have you lived in Southern England? __________


11. In which country were you born? _________________

12. What is the highest level of education you have completed?

( ) Secondary School (up to 16 years of age)  ( ) A levels (up to 18 years of age)
( ) University Degree Course  ( ) Other Qualification (please give details)

13. Do you smoke? ( ) Yes ( ) No
(If yes, please give details)

Number of cigarettes per day: _______  Number of years smoking: _______
Age at which started smoking: _______

14. Do you drink alcohol? ( ) Yes ( ) No
If yes, how many units per week: ____________________
(please see below for a guide on number of units of alcohol in common drinks).

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Measure</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary strength lager (4%) <em>(e.g. Carling, Fosters)</em></td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Strong lager (5.2%) <em>(e.g. Stella Artois, Kronenburg)</em></td>
<td>Pint</td>
<td>3</td>
</tr>
<tr>
<td>Strong lager <em>(e.g. Stella Artois, Carlsberg Export, Grolsch)</em></td>
<td>440ml can</td>
<td>2.2</td>
</tr>
<tr>
<td>Beer/ordinary strength Ale <em>(e.g. John Smith’s, Guinness)</em></td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Red/White Wine</td>
<td>Std 175ml</td>
<td>2</td>
</tr>
<tr>
<td>Red/White Wine</td>
<td>Lg. 250ml</td>
<td>3</td>
</tr>
<tr>
<td>Spirits</td>
<td>Std 25ml</td>
<td>1</td>
</tr>
<tr>
<td>Spirits</td>
<td>Lg. 35ml</td>
<td>1.4</td>
</tr>
<tr>
<td>Alcopop e.g. Smirnoff Ice, Bacardi Breezer, Reef</td>
<td>275ml</td>
<td>1.5</td>
</tr>
</tbody>
</table>

15. Have you ever broken / fractured any bones? ( ) Yes ( ) No ( ) Not sure

16. When you go outdoors, which body parts are usually exposed?

( ) Face only  ( ) Hands and face + arms and/or legs
( ) Hands and face  ( ) Hands and face + arms and/or legs + some/all of the torso
17. When you are out in the sun do you wear sunscreen (this can be at any time of the year)?

( ) Yes ( ) No

If yes, please specify the factor of sunscreen (SPF) used when at home (in the UK) and on holiday (holiday can include beach holidays or skiing holidays):

At home: ___________________ On holiday: ___________________

18. Do you have the habit of natural sunbathing? ( ) Yes ( ) No

If yes, please state how often you sunbathe.

( ) At least once a week ( ) At least twice a month
( ) Once a month ( ) Less than 6 times per year
( ) More than 6 times per year ( ) Occasionally

19. Do you use sunbeds?

( ) Yes ( ) No

If yes, when was the last time? ________ weeks ago

20. Where does your skin fit in?

( ) Type I - always burns, never tans (pale white).
( ) Type II - usually burns, tans with difficulty (white)
( ) Type III - sometimes mild burn, tans uniformly (cream white)
( ) Type IV - burns minimally, always tans well (moderate brown)
( ) Type V - very rarely burns, tans very easily (dark brown)
( ) Type VI - Never burns, never tans (deeply pigmented dark brown to darkest brown)

21. Do you usually do physical activities? ( ) Yes ( ) No

What do you do as an exercise? ____________________________________________

How often? ________

22. How familiar are you with the importance of having enough vitamin D in the body?

(not familiar at all)  0  1  2  3  4  5  6  7  8  9  10  (very familiar)
23. In your own words, what is vitamin D needed for, who needs it and how much?
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

24. Which, if any, of the following are a source of vitamin D? (tick any that apply)
(   ) Red meat  (   ) Cheese  (   ) Cod liver oil  (   ) Milk  (   ) Oily fish
(   ) Vegetables  (   ) Sunlight  (   ) Eggs  (   ) Don’t know
(   ) Chicken  (   ) White fish  Other: ________________

25. Are you currently taking any of the following supplements? (tick any that apply)
(   ) Vitamin D (go to Question 6)  (   ) Fish Oil with Vitamin D (go to Question 6)
(   ) Calcium with Vitamin D (go to Question 6)  (   ) Cod Liver Oil (go to Question 6)
(   ) Multi-Vitamin with Vitamin D (go to Question 6)  (   ) None

26. If no, have you taken any of the following supplements within the last year? (tick any that apply)
(   ) Vitamin D  (   ) Fish Oil with Vitamin D  (   ) Calcium with Vitamin D
(   ) Cod Liver Oil  (   ) Multi-Vitamin with Vitamin D  (   ) None (go to Question 9)

27. How frequently do/did you take this? (tick one option only)
(   ) Less than once a month  (   ) Once a month  (   ) Once every 2 weeks
(   ) Once a week  (   ) 2 - 3 times a week  (   ) 4 - 6 times a week
(   ) Daily  (   ) Other: __________________________

28. If known, what supplement do/did you take and what dose of vitamin D does/did it provide?
Supplement: ________________________________  Dose: __________________
29. Was the supplement prescribed by your GP or other Health Professional? (   ) Yes (   ) No

30. Have you ever had your vitamin D status measured? (   ) Yes (   ) No
If yes, what was the reason for the test and what was the result (if known)?
Reason: __________________________________________________________
Results: __________________________________________________________

31. How much milk do you have per day?
(   ) Never (   ) Less than ½ pint (   ) ½ pint (   ) 1 pint (   ) More than 1 pint

32. How often do you eat the following per week:
Eggs (   ) Never (   ) less than once (   ) once (   ) 2-5 times (   ) + 5
Oily fish* (   ) Never (   ) less than once (   ) once (   ) 2-5 times (   ) + 5
*such as sardines, tuna, salmon, mackerel, herring, kippers, trout and pilchards.
Liver (   ) Never (   ) less than once (   ) once (   ) 2-5 times (   ) + 5

Thank you for taking time to answer our questionnaire. Your help is greatly appreciated!

IF YOU HAVE ANY QUESTIONS, PLEASE CONTACT

Miss Marcela Mendes (m.moraesmendes@surrey.ac.uk) telephone (01483) 689222.
An answering machine will pick up any calls if we are unavailable.

If you have any concerns about the study please contact Dr Susan Lanham-New
(s.lanham-new@surrey.ac.uk), telephone (01483) 686476.