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).

Principal Investigator:
Name: Miss Marcela Moraes Mendes (PhD researcher)
Address: Department of Nutritional Sciences, Faculty of Health & Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH
Telephone: 01483 689222
Email: m.moraesmendes@surrey.ac.uk

PhD Supervisor:
Name: Professor Susan Lanham-New
Address: Department of Nutritional Sciences, Faculty of Health & Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH
Telephone: 01483 6896476
Email: s.lanham-new@surrey.ac.uk

Co-Supervisors:

Name: Dr Kathryn Hart (Co-supervisor)
Address: Department of Nutritional Sciences, Faculty of Health & Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH
Telephone: 01483 68 6438
Email: k.hart@surrey.ac.uk

Name: Dr Laura Tripkovic (Co-supervisor)
Address: Department of Nutritional Sciences, Faculty of Health & Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH
Telephone: 01483 686429
Email: laura.tripkovic@surrey.ac.uk

Name: Dr Patricia Borges Botelho (Collaborative supervisor)
Address: Faculty of Nutrition, Federal University of Goiás, Goiânia, Goiás, Brazil 74605-080
Telephone: +55 (62) 3209-6270
Email: patriciaborges.nutri@gmail.com

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. (Uinterlinden 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 divulgating 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.
Facebook pages we intend to target :
Science Without Borders UK students: https://www.facebook.com/groups/489494047812445/ and https://www.facebook.com/groups/csf2014/
Brazilians in London: https://www.facebook.com/groups/301278086667948/
Brazilian Students in the UK Association (ABEP): https://www.facebook.com/ABEPUK/?fref=ts
General Consulate of Brazil in London: https://www.facebook.com/cglondres/info/

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.
Facebook pages we intend to target:
Federal University of Goias: https://www.facebook.com/universidadefederaldegoias/?fref=ts
Faculty of Nutrition, Federal Uni of Goias: https://www.facebook.com/fanut.ufg.5?fref=ts
Faculty of Nutrition Postgrads, Federal Uni of Goias:
https://www.facebook.com/mestrado.fanut.7?fref=ts

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 (osteoporosis 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 theses 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 trail 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: Marcela M Mendes **Date:**
Miss Marcela Moraes Mendes 17 January 2016

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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:

	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: …….
   ……

4. Do you regularly take vitamin supplements containing vitamin D?

   Yes No Don't know

   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)? Yes No

   If yes, please provide details: ……..

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……..
   Month/year……..
   Country of visit……..
   Month/year……..

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……..
   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: ___/___/_____