



**The Inter-relationship between Vitamin D: Bone, Illness,
Injury and its Impact on Exercise Performance**
(The D-Biicep Study)

Study personnel and contact details

Principal investigator:

Professor Susan Lanham-New
Department of Nutritional Sciences
Faculty of Health & Medical Sciences,
University of Surrey, Stag Hill campus
Guildford, Surrey GU2 7XH

Telephone: 01483 6896476

Email: s.lanham-new@surrey.ac.uk

Co-investigators:

Miss Saskia Wilson-Barnes (PhD student)
Department of Nutritional Sciences
Faculty of Health & Medical Sciences
University of Surrey, Guildford, Surrey GU2 7XH

Telephone: 01483 689222

Email: s.wilson-barnes@surrey.ac.uk

Dr Julie Hunt
Department of Nutritional Sciences
Faculty of Health & Medical Sciences
University of Surrey, Manor Park Campus
Guildford, Surrey GU2 7WG

Telephone: 01483 689400

Email: j.hunt@surrey.ac.uk

Dr Ralph Manders
Department of Nutritional Sciences
Faculty of Health & Medical Sciences
University of Surrey, Manor Park Campus
Guildford, Surrey GU2 7WG

Telephone: 01483 688668

Email: r.manders@surrey.ac.uk

Synopsis

Title	The Inter-relationship between Vitamin D: Bone, Illness, Injury and its Impact on Exercise Performance
Acronym	<i>The D-BIICEP Study</i>
Chief Investigator	Professor Susan Lanham-New
Objectives	To explore the inter-relationship between vitamin D status, calcium homeostasis, bone health, injury, illness and sport performance in young active adults.
Study Configuration	Assessment of vitamin D status, muscular strength, endurance capacity, body composition and bone mineral density in healthy university level athletes before and after 20 week of competitive training and non-trained control subjects before and after 20 weeks.
Setting	The Clinical Investigative Units (CIU), Department of Nutritional Sciences, University of Surrey Surrey Human Performance Institute (SHPI), Surrey Sports Park, Guildford
Sample size estimate	Sample size calculation is based on detecting a mean difference in 25 (OH) D status (study primary endpoint) between indoor and outdoor athletes, considering the standard deviation (13.2 ng.mL) and effect size (0.48) reported previously (Halliday et al 2011). To give 80% power and a significance level $P < 0.05$ the study will be conducted with 18 participants in each arm. To allow for 15% dropout 63 participants will be recruited
Number of participants	63 subjects; 21 trained males, 21 trained females and 21 controls(male and female)
Eligibility criteria	Males or females aged 18-30 that regularly partakes in training/competition for a sport competing in BUCs (≥ 4 hrs/ week) will be eligible to enroll in the trained group. The untrained control group must not exceed 150 min/wk of physical activity. All subjects need to be in good physical health and should have a BMI >18 kg/m ² and able to provide written informed consent.
Description of interventions	Two test sessions in which Vitamin D concentration, muscular strength and power, aerobic capacity, bone mineral content and body composition will be assessed. This will be followed by 20 weeks of normal training and competition. After this 20wk period the same two test sessions will be repeated.
Duration of study	~24 weeks
Outcome measures	Vitamin D concentration (25(OH)D Status) Handgrip and knee extension isometric strength Aerobic endurance capacity (VO _{2 max}) Body composition, including total and regional assessments of lean and fat mass. Bone mineral content of the Tibia.

	Whole body and hip/femur bone mineral density.
Statistical methods	Descriptive data (Vit D, Strength, VO _{2 max} , fat, lean and bone mass) will be expressed as the group mean (\pm SD) or as the median (inter-quartile range) if the data are non-parametric. Comparison of pre- and post-training periods will be made using ANOVA.

Study background information and rationale

It is known that poor vitamin D status is a very common problem in the UK, specifically within the athletic community (Owens et al., 2014). It is also known that a lack of vitamin D availability has potentially serious health implications, especially with respect to bone and muscle function (Holick et al., 2005).

The risk of vitamin D deficiency or insufficiency among athletes is thought to depend largely on geographical location and type of sport (indoor vs. outdoor) (Halliday et al., 2011) but there is limited data on young adults within the United Kingdom partaking in University Sport. In addition to this there has been little research focussing upon the relationship between sport performance and vitamin D status. In line with this, little is known of the effects of different sports on bone health between the sexes. Weight bearing exercises such as rugby and basketball evoke very different mechanical loading profiles on the bone compared to non-weight bearing exercises such as swimming and can therefore also play an instrumental role in bone health in these physically active populations. Previous research has also failed to provide definitive conclusions upon the effect of Vitamin D status on bone, illness, injury and sport performance within the UK as most research has been at lower latitude (Forney et al., 2014).

A control group will be included into this study to explore whether young adults are also at significant risk of Vitamin D insufficiency or deficiency. This is because there is limited data on this population group. Therefore, the results obtained from this study will provide vital information on UK university populations and their vitamin D status. This will help to inform the wider scientific community to determine future public health strategies and thus potentially positively impacting on the health of the young adult sporting population and sedentary controls for years to come.

Study objectives and hypothesis

Primary objective

To determine the effects of regular training and competition on the inter-relationship between vitamin D status, calcium homeostasis, bone health, injury, illness and sport performance in young active adults.

Secondary objective

- i) To measure bone mineral density and content in winter and spring, and its relationship with Vitamin D status and the incidence of injury in athletes.

- ii) To measure vitamin D status in winter and spring to determine its relationship with the incidence of illness and fitness outcomes.
- iii) To distinguish the effects of indoor/outdoor/mixed training and competition on vitamin D status.
- iv) To interpret the effects of high/medium/low impact sports on bone mass.
- v) To measure changes in health and skill related components of fitness (vertical jump, hand-grip strength, knee extensor muscular strength and aerobic fitness) throughout an academic sporting season.
- vi) To measure any incidence of injury and illness that occurs between winter and spring and its correlation with Vitamin D status, training and bone health.
- vii) To ascertain whether there are any differences in the research outcomes between trained and control subjects

Hypothesis

We propose that athletes will have a sub-optimal (<50 nmol/l) of 25 (OH) D status at baseline and a poor vitamin D status (lowered plasma 25(OH)D) during the spring as a result of lower daylight exposure during the winter period. In addition to this we assume that those who regularly compete and train indoors will have a lower Vitamin D status at baseline due to insufficient exposure to ultraviolet B radiation from the Sun.

Study design

The D-BIICEP Study is an observational study. Participants will be assessed at baseline in winter (September/October) and again at the end of the observational period in spring (March/April).

Outline of Study

- **Pre-screening:** screening questionnaire is administered to participant.
- **Session 1:** Consent forms to be signed. pQCT test and weight, height and body composition. Hip and femoral head DEXA scan. Jump height and maximal oxygen uptake ($VO_{2\max}$) test.
- **Session 2:** Plasma samples collected and muscular strength tests performed.

20 weeks of training/ competition for their respective University Sport Team or regular physical activity levels for the control subjects.

Participants are expected to complete their Illness and Injury Questionnaire

- **Session 3:** pQCT test, weight, height and body composition. Hip and femoral head DEXA Scan. Jump height and maximal oxygen uptake ($VO_{2\max}$) test.
- **Session 4:** Plasma samples collected and muscular strength tests performed

Trial Visit Activities

During this study the subjects will be asked to visit the labs on two occasions, at the beginning of the study for baseline measurements, and again on two occasions at the end of the study.

At both trial visits, participants will have the following outcomes assessed:

- Sport performance: Vertical jump height, muscular strength and aerobic fitness
- Peripheral Quantitative Computed Tomography (pQCT) scan of the bone mineral composition of the tibia.
- Total and %age lean and fat mass and total bone mineral density via Dual Energy X-ray Absorptiometry (DEXA).
- Hip and femoral head bone mineral density and content to assess fracture risk via DEXA.
- Serum 25(OH)D levels, lipid profile, glucose, insulin, serum calcium, albumin, parathyroid hormone, C-terminal telopeptide (CTX), full blood count, kidney, thyroid and liver function (≈15ml of whole blood will be collected for these measurements).
- Dietary intake using self-reported food diaries
- Illness and injury incidence will recorded daily throughout the study in a booklet provided to the participants.

Aerobic fitness will be tested using a VO_2 max exercise protocol using a stationary cycle ergometer, the participants will be expected to perform this to maximal effort or exhaustion. The test will consist of progressive increments in cycling workload (power output, W) until volitional fatigue. The test will take about 8 – 12 minutes to complete and is dependent upon the fitness of the participants. This will take place in the Sport & Exercise Science laboratories based in the Clinical Investigation Unit (22AX00). All subjects will be supervised during exercise at all times and will implement an adequate warm-up and cool-down to minimize the risk of injury. Staff will be on hand throughout the testing trained adequately in basic life support (including defibrillation).

Muscular strength assessments will be performed at the Surrey Human Performance Institute. Muscular strength of the knee extensor and handgrip muscles will be determined using an Isokinetic Dynamometer and a handgrip dynamometer respectively. For each assessment three maximal effort isometric contractions will be performed and peak torque (nM) and strength (kg) will be recorded. Muscular power will be assessed through performance of a countermovement jump (CMJ).

Body composition (absolute and relative amount of lean and fat mass), whole body bone mineral density and hip and femur bone mineral density will be measured with the use of DEXA (located in the Sport & Exercise Science laboratories based in the Clinical Investigation Unit). Two scans will be performed: one for the assessment of whole body bone mineral density and body composition, and the other scan is performed to specifically assess fracture risk by scanning the hip and femoral head. Effective exposure doses for these scans are $\sim 8\mu\text{Sv}$ and $\sim 4\mu\text{Sv}$ respectively. The total effective dose is depending on the size ('thickness' & height) of the volunteer and is approximately equivalent to the cosmic radiation exposure received in 45min of a transatlantic flight, or the radiation dose received on eating 80g of brazil nuts (Health Protection Agency).

Bone mineral content of the tibia will be assessed using pQCT and this will be correlated to bone mineral density data obtained from DEXA. One scan will be performed and effective exposure doses will be between 1.5-1.8 μ Sv which is comparable to 10min on a transatlantic flight or one handful of brazil nuts. The estimated total exposure dose for a single subject during the entire study is ~28 μ Sv which is the same level of exposure as an average UK citizen is exposed to over the course of 4 days via background radiation.

Results of the body composition, bone mineral density, jump height, muscular strength, aerobic fitness (maximal oxygen uptake: VO₂max) 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.

Upon commencing the study, the participants will be required to track their incidence of illness and training habits during the 20-week experimental period between baseline and conclusion measurements. Throughout this period they must record the incidence of illness and days trained during the months in a check-list booklet, which will be provided to them. An email reminder will be sent to prompt all participants to complete these booklets on a daily basis during the experimental period, this should only take 1-2 minutes every evening. Throughout the duration of the trial, the participants will be contacted via telephone/ email on a monthly basis to discuss any issues and maintain good communication.

Recruitment

Participants will be recruited from the University of Surrey; posters will be positioned around the Stag Hill campus, Manor Park Campus and Hazel Farm. Flyers will be placed in communal areas, the students' union, the university library and at Surrey Sports Park to promote awareness for the study. In addition to this, a brief presentation will be given to students following their lectures in the same format as the poster or flyer.

Coaching staff and the signatories of Team Surrey committees will also be approached for the study and flyers will be provided to hand out to their team members if they are keen to complete this as a team. 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.

If the individual is interested in participating in the study after reading the information sheet, they will contact the investigator and session 1 will be arranged. A member of the research team will inform the participant of all aspects pertaining to participation in the study before consent is sought.

Children, and other vulnerable groups, including those unable to provide informed consent will not be recruited. Moreover, we do not have access to translator or interpreter services, so will not recruit those who do not have a good understanding of the English language either in written or oral form.

It will be explained to the potential participant that entry into the study is entirely voluntary and that they can withdraw at any time. In the event of their withdrawal it will be explained

that their data collected so far could be erased, but we will seek their consent to use the data collected up to the point of withdrawal in the final analyses where appropriate.

University Athlete Inclusion criteria

- Male or female
- Aged 18-30
- Regularly train/compete for Team Surrey Basketball, Boat Club, Swimming and Waterpolo, Netball or Rugby (for more than 4 hours/week)
- In good physical health
- Have a BMI $>18 \text{ kg/m}^2$
- Able to provide written informed consent

Control Group Inclusion Criteria:

- Male or Female
- Age 18-30
- Do **not** use sun beds/ will not be going on a sun holiday/ returning home to a traditional sun holiday location between February 2017 and October 2017. This is because the results will be used to represent the Vitamin D status of young sporting individuals residing within the UK during these seasons.
- Do **not** take supplements containing Vitamin D
- Do **not** compete for Team Surrey and exercise less than 150 minutes / week.
- BMI: 18 – 30 kg/m^2

Exclusion criteria

- Currently receiving treatment for medical conditions that are likely to affect vitamin D metabolism
- Hypercalcaemia ($>2.5 \text{ mmol/L}$)
- Regular use of sun-beds
- Having a sun holiday one month prior to commencing the study or plans for a sun holiday within the study period or will be returning home to a sun holiday location.
- 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).
- Excess alcohol intake for participants aged 18 years (> 21 units for males, > 14 units for females per week, as per Government guidelines)
- Those under dietary restriction (except vegetarianism) or following a weight-reducing diet.
- Clinically significant haematological abnormalities other than mild anaemia ($\text{Hb} < 12.0 \text{ g/dl}$)
- Active malignancy
- 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.

Participant 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. If the participant suffers an serious adverse event

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 15% has been accounted for in the recruitment targets.

Informed consent

All participants will provide written informed consent for participation, and use and retention of the study data. The Consent Form will be signed and dated by the participant before they enter the study. The Investigator will explain the details of the study and provide a Participant Information Sheet and a DEXA information folder, ensuring that the participant has sufficient time to consider participating or not. The Investigator will answer any questions that the participant has concerning study participation.

Informed consent will be collected from each participant before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the participant and one will be kept by the Investigator.

Those incapable of providing valid consent will not be recruited. Participants under the age of 18 will not be recruited.

Statistics, Sample size and justification

Statistical analysis based on preliminary data from our group and Halliday et al (2011) indicates that a minimum of 52 subjects is required in order to detect a 35% difference in vitamin D concentrations across seasons at a power of 80%, significance level $P < 0.05$.

In order to achieve an equal division between genders, 26 males and 26 females from different indoor and outdoor sports across Team Surrey need to be recruited. Based on our preliminary study we are expecting a 15% drop out. We will therefore aim to recruit 63 subjects (21 females, 21 males and 21 sedentary controls, males and females).

Descriptive data ((25(OH)D status, jump height, muscular power, muscular strength, $VO_{2\text{ max}}$, fat, lean and bone mass) will be expressed as the group mean (\pm SD) or as the median (inter-quartile range) if the data are non-parametric. Comparison of pre- and post-training periods will be made using ANOVA.

Quality assurance & audit

Insurance and indemnity

The University of Surrey has taken out an insurance policy to provide indemnity in the event of a successful litigious claim for proven non-negligent harm.

Study conduct

The study coordinator/local investigator, or where appropriate, a nominated designee of the sponsor, shall carry out site and study audits.

Study data

Monitoring of study data shall include confirmation of informed consent prior to inclusion; calculating and checking prior exposure to ionising radiation; data storage and data transfer procedures; local quality control checks and procedures, including QA scans, and back-up of any local databases. A nominated designee of the study team, shall carry out monitoring of study data as an ongoing activity.

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.

Record retention and archiving

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Surrey Code of Research Conduct and Research Ethics, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

Statement of confidentiality

Medical or personal information obtained from participants as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above.

Medical information provided by participants may be given to any medical personnel involved in the study if required for purposes related to the safety or health of that participant.

Data generated as a result of this study will be available for inspection on request by the participants GP (with the participant's consent), the University of Surrey representatives, the REC, local R&D Departments and the regulatory authorities.

Publication and dissemination 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.

Participant stipends and payments

Participants will be paid specifically for participation in the study although an inconvenience allowance is usually offered for taking part in the study.

References

Forney,L.A., Earnest,C.P., Henagan,T.M., Johnson,L.E., Castleberry,T.J., *et al* (2014) 'Vitamin D status, body composition, and fitness measures in college-aged students', *Journal of strength and conditioning research / National Strength & Conditioning Association*, 28 (3), pp. 814-824.

Halliday,T.M., Peterson,N.J., Thomas,J.J., Kleppinger,K., Hollis,B.W., *et al* (2011) 'Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes', *Medicine and science in sports and exercise*, 43 (2), pp. 335-343.

Holick,M.F. (2005) 'The vitamin D epidemic and its health consequences', *The Journal of nutrition*, 135 (11), pp. 2739S-2748S.

Owens,D.J., Webber,D., Impey,S.G., Tang,J., Donovan,T.F., *et al* (2014) 'Vitamin D supplementation does not improve human skeletal muscle contractile properties in insufficient young males', *European journal of applied physiology*, 114 (6), pp. 1309-1320.