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**The relationship between vitamin D status and muscle strength in young healthy adults from sunny climate countries currently living in northeast of Scotland**

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Nor Aini Jamil, Stuart Gray, William Fraser, Shona Fielding and Helen Macdonald declare that they have no conflict of interest.

## **Abstract**

### *Summary*

The current study examined the relationship between vitamin D status and muscle strength in young healthy adults: residents (> 6 months) and newcomers (0-3 months), originally from sunny climate countries but currently living in the northeast of Scotland. Our longitudinal data found a positive, albeit small, relationship between vitamin D status and knee extensor isometric strength.

### *Introduction*

Vitamin D has been suggested to play a role in muscle health and function, but studies so far have been primarily in older populations for falls prevention and subsequent risk of fractures.

### *Methods*

Vitamin D status was assessed in a healthy young adults from sunny climate countries (n=71, aged 19-42 years) with 56% seen within 3 months of arriving in Aberdeen [newcomers; median (range) time living in the UK = 2 months (9-105days)] and the remainder resident for > 6 months [residents; 23 months (6-121 months)]. Participants attended visits every 3 months for 15 months. At each visit, fasted blood samples were collected for analysis of serum 25-hydroxyvitamin D [25(OH)D], parathyroid hormone (PTH), carboxy-terminal collagen crosslinks (CTX) and N-terminal propeptide of type I collagen (P1NP). Maximal voluntary contractions (MVC) were performed for grip strength (both arms) and for maximal isometric strength of the knee extensors (right knee).

### *Results*

There were small seasonal variations in 25(OH)D concentrations within the newcomers and residents, but no seasonal variation in bone turnover markers. There was a positive, albeit small, association between 25(OH)D and knee extensor maximal isometric strength. Mixed modelling predicted that for each 1 nmol/L increase in 25(OH)D, peak torque would increase by 1 Nm (p=0.04).

### *Conclusions*

This study suggests that vitamin D may be important for muscle health in young adults migrating from sunnier climates to high latitudes, yet the potential effect is small.

Keywords: vitamin D, muscle strength, ethnic, isometric, hand grip

## **Introduction**

As people from sunnier climate countries living in the West are at increased risk of vitamin D deficiency due to their pigmented skin and cultural practices (i.e., sun avoidance and clothing style), especially at high latitude, and if vitamin D is important for muscle health, this group may be particularly vulnerable to poor muscle function. There are limited randomised controlled trials (RCT) investigating the effect of vitamin D on muscle strength in people from sunnier climate countries visiting West countries [1]. Furthermore, no longitudinal data studying the relationship between vitamin D and muscle strength and how this relationship is affected by emigrating from low to high latitudes exists. Early evidence supporting a role of vitamin D in muscle health can be found in patients with rickets and osteomalacia, who develop myopathy and muscle weakness which is reversible with vitamin D supplementation [2-3]. Further support for a role of vitamin D in muscle came from the identification of vitamin D receptors in skeletal muscle tissue, although this remains controversial [4–6]. The association between muscle strength and vitamin D status has been explored mainly in older populations for falls prevention and subsequent risk of fractures, but little research has been performed in younger adults. A meta-analysis of 30 RCTs showed a small effect of vitamin D supplementation, especially on lower limb muscle strength, among people with 25-hydroxyvitamin D [25(OH)D] level <30 nmol/L [7]. This is of particular importance as population studies have shown that mid-life muscle strength is associated with long-term mortality risk, old age disability and longevity [8–10].

The aims of this longitudinal study were (1) to examine seasonal variation in vitamin D status and muscle strength among people from sunnier climate countries shortly after arriving in Aberdeen, UK (57°N) and (2) to quantify the relationship between vitamin D status and muscle strength.

## **Methods**

### *Participants*

Participants (age range 19-59) originated from sunny climate countries between 45°N and 45°S, who were currently visiting Aberdeen and intending to stay for one year or more. Potential participants were excluded if they reported having chronic gastrointestinal diseases associated with malabsorption, kidney disease, liver disease, bone-related diseases or pregnancy.

### *Study visits*

Participants were recruited at three different time points between 0 and 3 months of arrival in Aberdeen: (a) autumn 2012, (b) spring 2013 and (c) autumn 2014. A separate group who had been residing in Aberdeen more than 6 months were also included (recruited in winter 2012). Participants were then grouped as (1) autumn newcomers (those recruited in autumn 2012 and autumn 2013), (2) spring newcomers and (3) residents. Ethical approval for this study was granted by the College of Life Sciences and Medicine Ethics Review Board (CERB), University of Aberdeen. Written informed consent was obtained from all individual participants included in the study.

### *Number of participants recruited*

Based on RCT data of vitamin D among young healthy Asian Indians [11], where mean baseline dominant handgrip strength was 31.0 kg (SD=9.7 kg), recruiting 56 people would be sufficient to detect a true difference of 6.0 kg [12] (90% power and 5% significance). Allowing for a 30% drop-out, a total of 73 participants were required.

### *Study Protocol*

Participants attended visits at the Human Physiology Laboratory, University of Aberdeen, every 3 months for 15 months (5 visits in total), except the autumn 2013 cohort who had only 4 visits in total. During each visit the following measurements were made:

### *Blood Sampling*

Blood was drawn between 0700 and 1100 hours after a 10-hour overnight fast. Serum and plasma samples were separated by centrifugation at 3000 rpm, 4°C for 15 minutes, not more than 1 hour after sampling. Both serum and plasma were aliquoted into sterile eppendorfs, labelled and stored at -80°C. Each individual's samples were analysed in a single batch. Measurements for serum 25(OH)D, plasma parathyroid hormone (PTH), carboxy-terminal collagen crosslinks (CTX) and N-terminal propeptide of type 1 collagen (P1NP) were undertaken in the Department of Medicine, University of East Anglia, Norwich, UK. This laboratory is registered with the quality control group for vitamin D (DEQAS).

Serum 25(OH)D concentrations were measured by dual tandem mass spectrometry. The inter-assay coefficient of variation (CV) was <10% for both 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>, respectively. The sum of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> concentrations was taken as total 25(OH)D. For intact PTH, CTX and P1NP, plasma samples were analysed using an Elecsys electrochemiluminescent immunoassay (ECLIA) with a Cobas e501 analyzer (Roche Diagnostics, Indianapolis, USA). Inter/intra assay CV was <4% for PTH, <5% for CTX and <4% for P1NP. Vitamin D binding protein (VDBP) was measured using a monoclonal enzyme-linked immunosorbent assay (Human vitamin D BP Quantikine ELISA Kit; R&D Systems, Oxford, UK) according to the manufacturer's instructions. The inter/intra-assay CV was <7.4% and <6.2%, respectively. Two kits were used to analyse VDBP samples in spring and autumn. The kits were standardised using an identical analysis of 12 samples producing a linear equation:  $y$  (kit 1, spring) = 0.8252x (kit 2, autumn) + 1.012. Bioavailable 25(OH)D (BioD) and free 25(OH)D (FreeD) were calculated as described by Powe et al. [13]. Serum albumin concentration was not measured in the current study; all participants were assumed to have a normal albumin level of 43 g/L (using a reference range 38 g/L - 48 g/L showed a difference of 2%).

### *Hand grip strength*

After a standard breakfast (coffee/tea, biscuits and yogurt), maximal strength was measured during a maximal voluntary contraction (MVC) of the dominant and non-dominant arms with a T.K.K.5401 GRIP D digital grip dynamometer (Takei & Co, Ltd, Tokyo, Japan). In a standing position, participants were asked to extend their arms downwards and grip the dynamometer with

the middle phalanges between the fingers and the palm. They were then asked to squeeze with maximum force and sustain the effort for three to five seconds. Standardised verbal encouragement was given for each contraction. Strength was measured twice for each arm (60 seconds rest between contractions) and the highest force for each hand was taken and averaged to give an individual outcome (average maximum hand grip strength).

### *Knee strength*

The maximal isometric strength of the knee extensor muscles of the right leg was performed during an MVC using a Biodex dynamometer System 2 (Biodex Medical Systems, Model 820-202, Shirley, New York, USA). The axis of the knee joint was aligned with the center of rotation of the dynamometer arm and the lower leg was strapped to the lever arm just proximal to the ankle. Participants were restrained at the waist, shoulders and the distal part of the thigh, and the backrest was set at 100° from the horizontal base of the seat. Peak torque was assessed at a knee angle of 73°. Each MVC contraction lasted over 10 seconds with this repeated a minimum of three times, with 120 seconds rest in between each contraction. The highest value of the tests was used for analysis. Participants were verbally encouraged to perform at their maximum.

### *Skin colour*

Skin colour was measured on the face (average right and left cheekbones), and the inner and outer right forearm using a handheld chromameter (CM-2600d Spectrophotometer; Konica Minolta Photo Imaging, London [UK] Ltd). Calibration against a standard white tile was carried out before each set of measurements. Measurements were recorded for luminance or lightness ( $L^*$ ), the 'red-green' ( $a^*$ ) and the yellow-blue ( $b^*$ ) axes of colour based on the Commission International De L'Eclairage  $L^*a^*b^*$  system [14]. The individual typology angle (ITA) was calculated using the following equation:

$$ITA = [\arctangent (L^* - 50)/b^*] \times 180/\pi [15]$$

### *Sunlight exposure*

Body surface area (BSA) exposure, previous sun exposure and holidays abroad were assessed by a questionnaire adapted from a previous study [16]. At the end of each visit, participants were given two polysulphone film badges which measure sunlight exposure (UVB radiation) and were

instructed to wear the badges on their outdoor clothing for seven consecutive days (badge A for 4 days and badge B for 3 days). Clear instructions for wearing the badges were given to all subjects. The badges were read on a spectrophotometer (Perkin Elmer UV/VIS Lambda 2 Spectrophotometer) before and after use, at a wavelength of 330 nm. Once used, the badges were kept in a sealed envelope. Standard erythema dose (SED) was calculated using the following equation:

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

where  $\Delta A_{330}$  was the change in absorbance of the film badge pre- to post-UVB exposure [17-18].

#### *Dietary calcium and vitamin D intake*

Dietary calcium intake was assessed via a modified calcium intake questionnaire [19]. The modification included the addition of rice as the major cereal of diet in people from sunnier climate countries and excluded pancakes/crumpets. For dietary vitamin D intake, a short questionnaire which included the few foods containing vitamin D in the UK was used [20]. To consolidate the dietary assessment, foods from the calcium and vitamin D questionnaires were combined into a single questionnaire. Use of dietary supplements of calcium and vitamin D were assessed at the end of the questionnaire. Participants were asked to determine the frequency and amount of intake as well as the brand name for commercial products in a week during each season. Food portion sizes were determined by the food photograph atlas of UK and Malaysia [21-22] or household measurement. Each food from the questionnaire was assigned its calcium and vitamin D content (according to the pre-defined weight) using information from the 6<sup>th</sup> edition, McCance and Widdowsons' Composition Foods [23] and the Malaysian Food Composition [24] databases.

#### *Physical activity*

At the end of each visit, participants were given an Actigraph accelerometer (GT3X or GT3X+) (Actigraph, Inc, Pensacola, FL), fitted with an elastic belt. Participants were asked to wear the monitors on their waist for seven consecutive days during waking hours, except when bathing, showering or swimming. A similar setting of integrating the raw data into 60-second epochs was used for both models. The monitors were fully charged and initialised with the starting date (the

next day after the visit) and time (05:00) using ActiLife software version 6.6.3. The monitors continued recording the activity data until they were downloaded. Written instruction describing how to use the monitor was provided for each participant along with a simple log sheet for the participant to record when the monitor was worn and removed. A compliance check of wearing the device was performed using a previously derived algorithm [25]. Data were screened using the vertical magnitude and only data with a minimum of 3 valid days containing at least 10 valid hours per day were used. The participants' activity log sheets were used to check the accuracy of the wear-time algorithm and manual corrections were performed to ensure inclusion of only waking hours. The Freedson Adult VM3 (2011) cut points were used to determine vector magnitude counts per minute (VM CPM) [26].

### *Body weight and height*

Body weight was measured by a digital weighing scale (OHAUS, Model CD11-Eu, USA). The accuracy of this instrument was within 0.01 kg. The scale reading was checked at zero before the measurement, and participant stood on the centre of the scale without support, minimal clothing and the weight distributed evenly on both feet. Two measurements were taken and the average was calculated. The weight recorded was a fasted-weight (before blood collection) and measured at every visit.

For height measurement, a stadiometer (Holtain, UK) was used. The accuracy of this instrument was within 0.1 cm. Participants were asked to stand with feet together and the heels, buttock and upper part of back touching the wall. The participant's neck was placed in the Frankfurt plane. Two measurements were taken and the average was used for subsequent analysis. The height measurement was conducted during the baseline visit only.

### *Statistical Analysis*

All analyses were carried out using SPSS version 22. Data were checked for normality and when the distribution was skewed, the natural log was used to transform the data (body mass index, vector magnitude, knee isometric muscle strength, dietary calcium and vitamin D intake and 25(OH)D). Variables were described using mean and standard deviation or median and inter-quartile range as appropriate. Categorical variables described with number and percentage.

Comparisons between newcomers and residents were undertaken using an independent t-test or chi-square test as appropriate. To assess changes of vitamin D, hand grip strength and isometric knee strength, repeated measures ANOVA was performed for four visits (excluding the final visit) to maximise the total number of participants included in the analyses. The analyses were conducted separately for the three recruitment groups (autumn newcomer, spring newcomer and resident). Sensitivity analysis was carried out for Asians ethnicity only, as they were the largest group in this study. The mixed model was carried out to assess differences in the repeated measures (e.g. 25(OH)D levels) across groups while adjusting for additional important confounders and taking account of the correlation of the repeated measurements per individual. The final models only include independent variables which were significant at the 5% level.

## **Results**

### *Baseline participants' characteristics*

A total of 71 participants aged 19 to 42 years took part in this study, with retention rates of 90% at 12 months and 80% at 15 months (Figure 1). The majority of the participants were Asians (68%; 96% Southeast Asian), followed by Africans (14%) the Middle East (11%) and Caucasian/Hispanic (7%). Forty participants (53% female) were newcomers. The median time of residing in the UK was 2 months (range: 9-105 days) for newcomers and 23 months (range: 6-121 months) for residents.

There were no differences in baseline physical characteristics (Table 1). The newcomers had a significantly higher vitamin D status at baseline compared to the residents ( $p < 0.01$ ). Furthermore, more residents than newcomers had 25(OH)D concentration below 25 nmol/L at baseline ( $p = 0.01$ ), although the number of participants with baseline 25(OH)D levels above 50 nmol/L was small for both groups. After adjusting for the season of measurement at baseline, the spring newcomers had higher vitamin D status than residents in spring ( $p = 0.01$ ), but no differences in vitamin D status between the autumn newcomers and residents in autumn ( $p = 0.86$ ) was found.

### *Changes in vitamin D status*

There was seasonal variation in 25(OH)D concentrations within all recruitment groups (Table 2). Sensitivity analysis including only the Asian ethnicity also showed a seasonal variation in 25(OH)D concentrations within all groups. The difference between zenith (summer/autumn) and nadir (winter/spring) was small: 8 nmol/L, 6 nmol/L, and 9 nmol/L among the newcomers arriving in spring, autumn and the residents, respectively. There was significantly lower VDBP concentration, in all participants, in spring compared to autumn [mean (SD) spring = 4.2 (1.5)  $\mu\text{mol/L}$ ; autumn=4.5(1.6)  $\mu\text{mol/L}$ ,  $p<0.001$ ]. Seasonal changes in BioD and FreeD concentrations followed a similar pattern to the seasonal changes of total 25(OH)D concentrations (data not shown). No seasonal changes in PTH, P1NP and CTX levels were observed in this study (Table 3).

### *Muscle strength*

There was seasonal variation in hand grip strength among the autumn newcomers ( $p=0.03$ ) and residents ( $p=0.04$ ), but not among the spring newcomers (Table 2). Sensitivity analysis of the Asian ethnicity only however showed no seasonal variation in any recruitment groups. For isometric knee strength, both autumn newcomers and residents also showed seasonal variation (Table 2). This time, sensitivity analysis of the Asian ethnicity only showed seasonal variation in isometric knee strength among the Asian residents. In this group, the winter knee strength was lower than summer ( $p=0.003$ ).

### *Factors affecting vitamin D status*

The mixed model approach showed that ethnicity (Middle Eastern and African compared to Asian), timing (season) of visits, longer duration of time living in Scotland, increasing age and lighter skin colour were associated with lower 25(OH)D concentrations (Table 4). In contrast, Caucasian ethnicity, recent holiday in sunny location and total dietary vitamin D intake (including supplements) were significant positive predictors of 25(OH)D. Other potential variables, including season of recruitment, dietary calcium intake, body surface area exposed and daily SED were tested in the model, but were not statistically significant and therefore were removed from the final model (Model A). Sensitivity analysis was carried out: (a) excluding the

Caucasian/Hispanic ethnic group, (b) including Asian ethnicity only and (c) excluding high dietary vitamin D supplement intakes (above 800 IU/day). There was very little difference in the results compared to the first model and the effect sizes of the remaining factors were similar between the four models (data not shown).

#### *Factors affecting muscle strength*

Mixed models were constructed to investigate the association between 25(OH)D and hand grip strength (Table 5, Model B). There was no association between vitamin D status and hand grip strength ( $p=0.68$ ). The model predicted that African ethnicity, male, height and weight were associated with increased hand grip strength. Other potential variables such as season of recruitment, age, days spent in Scotland and physical activity measurements (i.e., vector magnitude and time spent in moderate physical activity) were tested in the model, but were not statistically significant and therefore were removed from the final model. The non-significant association between 25(OH)D and hand grip strength remained unchanged with the inclusion of VDBP as an independent variable (data not shown). BioD was also tested instead of total 25(OH)D as a predictor of hand grip strength but no significant association was found (data not shown).

The mixed model approach was also used to investigate the association between 25(OH)D and isometric knee strength (Table 5, Model C). There was a significant positive association between 25(OH)D and knee isometric strength. The model predicted that for each 1 nmol/L increase in 25(OH)D, peak torque increased by 1 Nm ( $p=0.047$ ). Other positive predictors of isometric knee strength include sex (male), weight and height. Ethnicity was found to be not significant in addition to other potential variables tested in the grip strength and was therefore excluded from the final model. Similarly, mixed model analyses were performed for knee isometric strength with the addition of VDBP or BioD (replacing total 25(OH)D) concentrations in Model C. Results showed that the effect size for 25(OH)D concentrations in predicting knee isometric strength was not improved with the inclusion of VDBP or BioD (Table 5, Model D and Model E).

## Discussion

This is the first longitudinal study to examine vitamin D status among newly arrived young adults from sunnier climate countries visiting northeast Scotland and a group of people from sunnier climate countries who had been resident for longer than 6 months. Our findings show that upon arrival in Scotland very few of the recent newcomers (n=5, 13%) had 25(OH)D concentrations above 50 nmol/L, a cutoff defined by the IOM as a sufficient level for bone health. Our findings of a small seasonal variation in 25(OH)D concentrations for Asians living in the north of Scotland in this study differs from previous observations of a lack of seasonal variation in 25(OH)D concentrations in people from the Indian subcontinent living in the south of England [27-28]. Yet, we found no seasonal variation in PTH, CTX and P1NP levels, which probably reflects the small magnitude of the seasonal variation in 25(OH)D concentrations. As previously showed by Darling et al., (2014), a large seasonal change in 25(OH)D concentration was associated with increased levels of serum PTH and CTX [29].

Within the limited number of observational studies among healthy young adults, our findings, with regards to 25(OH)D and muscle strength, are in line with a previous study in healthy men and women with a wider age range (20-76 years) [30]. Both the work of Grimaldi et al. and our study found a positive relationship between vitamin D status and isometric knee strength, and no association between 25(OH)D concentration and hand grip strength. Two other cross-sectional studies also reported no association between 25(OH)D concentration and hand grip strength [31-32]. Although Ceglia et al. found a positive association between 25(OH)D concentration and lower body physical function score (walking speed and chair stand) in an age-adjusted model, there was no association after adjusting for age and ethnic group and other multivariate-factors [31]. Marantes et al. found a significant association between the active form of vitamin D, 1,25(OH)<sub>2</sub>D, and knee isometric extension in women younger than 65 years [32]. Only one study among healthy young females demonstrated that 25(OH)D concentration was positively associated with hand grip strength in the dominant and non-dominant hand, but not with the lower limb muscle power as assessed by countermovement jump [33].

Our model indicated that a 20 nmol/L increase in 25(OH)D concentration is associated with a 20 Nm increase in knee extensor torque. Previous RCT studies with vitamin D supplementation among Asians showed an increase in 25(OH)D concentration in the treatment group [1,11,34]; but only one study with a high dose of supplementation (60,000 IU vitamin D/week plus calcium) found a positive effect of supplementation on muscle strength and muscle performance [11]. To distinguish whether the positive effect on muscle strength was due to calcium, vitamin D or the combination of both, the same research group conducted a different RCT study, but this time only among young females. The effect of vitamin D supplementation on muscle strength in females was not observed [34]. Further investigation of their earlier study found that the improvement in hand grip strength was only in males, suggesting a possible gender-specific difference [34]. With lower doses of vitamin D supplementation (400 IU/day and 1000 IU/day), no significant improvement in muscle strength was seen among immigrants with ethnic backgrounds of Middle Eastern, African and South Asian in Oslo (aged 18-50 years) [1]. It is worth noting that hand grip strength was used to assess muscle strength in all of these three RCTs, which has been found to be less sensitive in assessing changes in muscle strength and physical performance [7,35]. Different measures of muscle function (walking test, chair rising and jumping height) were performed for the lower limb muscles.

When we compare our findings to a meta-analysis of RCTs including all ethnic groups together, our longitudinal data reflect the small and positive association between vitamin D status and lower limb muscle strength [7]. The meta-analysis also found that, after vitamin D supplementation, a greater improvement in muscle strength was seen among people with at least a 25 nmol/L increase in 25(OH)D concentration. In the current study, very few of our participants had 25(OH)D concentrations greater than 50 nmol/L and yet an association between 25(OH)D and muscle strength was observed. In a previous RCT among immigrants in Oslo, it was also reported that even after supplementation, 62% (in 400 IU vitamin D group) and 43% (1000 IU vitamin D group) of the participants in the study did not reach serum 25(OH)D levels  $\geq$  50 nmol/L [1]. It is worth pointing out that the target of 50 nmol/L is primarily based on studies performed in Caucasians and some studies suggest that the association between 25(OH)D and health outcomes are different in other ethnic groups [36].

We also found seasonal difference in VDBP concentrations, with lower circulating VDBP in spring compared to autumn. No relationship between VDBP and muscle strength was observed. Others have demonstrated an association between VDBP genotypes and various health conditions including bone mineral density [37] and inflammatory bowel disease [38]. There have been concerns that for some ethnicities the monoclonal immunoassay does not detect all VDBP due to differences in VDBP genotype distribution, leading to an underestimate in total VDBP [39]. There are two separate ideas regarding the role of VDBP (1) high VDBP may influence the activity of vitamin D metabolites by limiting the biologic action of vitamin D, lending the ‘free hormone hypothesis’ (only the free fraction is available to exert metabolic effects in target cells) [13]; (2) VDBP bound to 25(OH)D may protect 25(OH)D against degradation, prolonging the half-life of 25(OH)D [40]. It is possible that the seasonal differences in VDBP could result in more 25(OH)D being bound in autumn when it is at a peak, and then less being bound in spring, releasing more free 25(OH)D as required. Clearly, further work is required to test this hypothesis.

To the best of our knowledge, the current study is the first longitudinal study in the UK to investigate vitamin D status and muscle strength in South East Asians. Blood samples were analysed in one batch in a laboratory registered with the vitamin D external quality assessment scheme (DEQAS) with the exception of VDBP measurement, where although samples were not analysed at the same time, they were adjusted for the different kits used. A standard protocol for muscle strength and other tests were closely adopted and importantly, they were supervised by the same researcher. The main limitations of our study include the relatively small sample size, the fact that multi-ethnic groups were recruited due to low number of people available for a single ethnicity and that a monoclonal antibody was used for measurement of VDBP.

## **Conclusion**

This is the first longitudinal study to investigate vitamin D status and muscle strength in young adults migrating from sunnier climates to high latitudes. Our data of positive, albeit small, relationship between vitamin D and lower limb muscle strength are in line with the current meta-analysis of RCT studies including all ethnic groups. If extrapolated to higher 25(OH)D increases, the equivalent change in muscle strength could be considered clinically meaningful, assuming

the relationship is causal and linear. The seasonal change in VDBP could indicate a mechanism by which the half-life of 25(OH)D could be extended, and more free 25(OH)D released when required.

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Table 1: Baseline characteristics and comparison between resident status

	<b>Newcomer (n=40)</b>	<b>Resident (n=31)</b>		<b>All (n=71)</b>
<b><u>Ethnic group (n,%)</u></b>				
Asian	29 (72.5)	19 (61.3)		48 (67.6)
Middle Eastern	3 (7.5)	5 (16.1)		8 (11.3)
African	6 (15.0)	4 (12.9)		10 (14.1)
Caucasian/Hispanics	2 (5.0)	3 (9.7)		5 (7.0)
<b><u>Gender (n,%)</u></b>				
Male	19 (47.5)	11 (35.5)		30 (42.3)
Female	21 (52.5)	20 (64.5)		41 (57.7)
<b><u>Physical characteristics (mean, SD)</u></b>				
Age (years)	27.4 (6.0)	30.2 (6.8)	<b>p</b> 0.07 <sup>a</sup>	28.6 (6.5)
Weight (kg)	65.9 (14.9)	67.5 (14.6)	0.56 <sup>a</sup>	66.6 (14.7)
Height (cm)	166.4	166.7 (9.2)	0.79 <sup>a</sup>	166.5 (8.7)
BMI (kg/m <sup>2</sup> ) <sup>†</sup>	23.1 (5.4)	23.9 (4.5)	0.56 <sup>b</sup>	23.4 (4.8)
BMI above 30 kg/m <sup>2</sup> (n,%)	3 (9.7%)	2 (5.0%)		5 (7%)
Vector magnitude (cpm) <sup>†</sup>	650 (225)	537 (193)	0.42 <sup>b</sup>	592 (222)
Hand grip strength (kg)	31.8 (10.4)	30.1 (10.4)	0.49 <sup>a</sup>	31.0 (10.4)
Knee isometric muscle strength (Nm) <sup>†</sup>	135.5 (41.2)	124.7 (37.3)	0.45 <sup>b</sup>	130.9 (39.7)
Dietary calcium intake (mg/day) <sup>†</sup>	535 (413)	502 (344)	0.17 <sup>b</sup>	533 (332)
Dietary vitamin D intake (IU/day) <sup>†</sup>	110 (134)	113 (155)	0.82 <sup>b</sup>	113 (135)
<b><u>Vitamin D status</u></b>				
25(OH)D (nmol/L) <sup>†</sup>	33.5 (18.0)	24.1 (14.8)	<0.01 <sup>b</sup>	28.8 (20.5)
<b>25(OH)D classification (n,%)</b>				
25(OH)D < 25 nmol/L	9 (22.5)	16 (51.6)	0.04 <sup>c</sup>	25 (35.2)
25(OH)D between 25 nmol/L and 50 nmol/L	26 (65.0)	13 (41.9)		39 (54.9)
25(OH)D > 50 nmol/L	5 (12.5)	2 (6.5)		7 (9.9)
<b>25(OH)D, adjusted for season of recruitment<sup>†</sup></b>				
Autumn	33.0 (22.1)	32.9 (15.5)	0.86 <sup>b</sup>	
Spring	33.9 (11.2)	22.5 (20.0)	0.01 <sup>b</sup>	

<sup>†</sup>Median, IQR. <sup>a</sup>: Independent t-test; <sup>b</sup>: Independent t-test using natural log-transformed data; <sup>c</sup>: Chi-square

Table 2: Seasonal changes in 25-hydroxyvitamin D and muscle strength according to recruitment group

	Autumn	Winter	Spring	Summer	Autumn	Winter	ANOVA (p)
<b>25(OH)D (nmol/L) (Median, IQR)</b>							
Autumn newcomer (n=20)	40.5(18.5)	31.9(18.3)	32.6(21.9)	36.6(27.8)			0.001 <sup>a</sup>
Asian, autumn newcomer (n=13)	36.5(18.0)	28.3(17.2)	30.1(15.6)	35.7(23.6)			0.001 <sup>a</sup>
Spring newcomer (n=13)			33.9(9.8)	45.5(24.7)	28.4(14.3)	23.1(7.7)	<0.001 <sup>a</sup>
Asian, spring newcomer (n=10)			34.4(11.2)	41.7(28.7)	27.7(16.0)	22.9(11.1)	0.003 <sup>a</sup>
Resident (n=27)		21.2(14.7)	21.6(11.5)	29.1(16.3)	32.5(11.3)		<0.001 <sup>a</sup>
Asian, resident (n=17)		20.0(14.5)	23.0(8.3)	27.9(7.6)	30.1(12.5)		0.002 <sup>a</sup>
<b>Hand grip strength (kg) (Mean, SD)</b>							
Autumn newcomer	29.9(7.7)	31.5(8.5)	31.1(9.0)	31.1 (8.8)			0.025
Asian, autumn newcomer	28.3(7.3)	29.6(8.0)	29.5(9.1)	28.9(8.5)			0.153
Spring newcomer			35.1(14.2)	36.4(13.8)	36.7(14.2)	36.4(14.3)	0.099
Asian, spring newcomer			30.7(10.2)	32.1(9.5)	32.1(9.4)	31.5(9.5)	0.090
Resident		29.8(10.0)	30.9(11.3)	31.5(11.1)	31.8(10.7)		0.035
Asian, resident		28.1(9.7)	28.9(10.8)	30.0(10.5)	29.9(10.5)		0.147
<b>Isometric knee strength (Nm) (Median, IQR)</b>							
Autumn newcomer	109.6(42.3)	124.5(51.5)	135.3(45.6)	132.3(39.6)			<0.001 <sup>a</sup>
Asian, autumn newcomer	102.8(59.8)	101.0(53.4)	127.5(47.7)	115.6(45.8)			0.091 <sup>a</sup>
Spring newcomer			141.2(72.9)	164.2(96.2)	143.0(88.2)	128.4(80.3)	0.117 <sup>a</sup>
Asian, spring newcomer			141.2(72.2)	164.2(94.1)	122.2(91.2)	128.4(80.1)	0.050 <sup>a</sup>
Resident		118.3(47.7)	132.6(57.8)	130.8(55.4)	130.5(59.0)		<0.001 <sup>a</sup>
Asian, resident		111.4(55.1)	129.0 (76.9)	130.8(62.7)	119.8(68.9)		0.003 <sup>a</sup>

<sup>a</sup>: Repeated measures ANOVA using natural log-transformed data.

Table 3: Seasonal changes in bone turnover markers according to recruitment group

	Autumn	Winter	Spring	Summer	Autumn	Winter	ANOVA (p)
<u>PTH (pg/ml)</u>							
Autumn newcomer <sup>†</sup> (n=20)	3.8 (2.7)	4.5 (1.7)	4.4 (2.9)	4.3 (2.1)			0.171 <sup>a</sup>
Asian, autumn newcomer <sup>†</sup> (n=14)	4.4 (2.9)	4.7 (1.6)	4.3 (1.8)	4.1 (2.3)			0.353 <sup>a</sup>
Spring newcomer <sup>†</sup> (n=14)			4.9 (2.7)	5.2 (3.0)	5.3 (2.4)	5.8 (4.0)	0.081 <sup>a</sup>
Asian, spring newcomer <sup>†</sup> (n=11)			5.1 (2.1)	4.9 (2.9)	5.3 (1.8)	5.5 (3.8)	0.094 <sup>a</sup>
Resident <sup>†</sup> (n=26)		5.1 (2.4)	5.2 (2.8)	4.8 (2.6)	5.9 (2.2)		0.172 <sup>a</sup>
Asian, resident <sup>†</sup> (n=16)		4.9 (2.9)	5.3 (4.5)	4.9 (3.8)	6.0 (2.1)		0.369 <sup>a</sup>
<u>CTX (µg/L)</u>							
Autumn newcomer <sup>†</sup>	0.46 (0.29)	0.40 (0.26)	0.38 (0.27)	0.39 (0.30)			0.288 <sup>a</sup>
Asian, autumn newcomer <sup>†</sup>	0.42 (0.24)	0.43 (0.29)	0.37 (0.28)	0.35 (0.33)			0.445 <sup>a</sup>
Spring newcomer <sup>‡</sup>			0.39 (0.15)	0.39 (0.16)	0.41 (0.15)	0.35 (0.15)	0.118
Asian, spring newcomer <sup>‡</sup>			0.41(0.15)	0.41 (0.17)	0.43 (0.15)	0.37 (0.16)	0.308
Resident <sup>‡</sup>		0.32 (0.12)	0.33 (0.14)	0.33 (0.14)	0.34 (0.12)		0.563
Asian, resident <sup>‡</sup>		0.33 (0.12)	0.34 (0.14)	0.34 (0.14)	0.35 (0.13)		0.798
<u>P1NP (µg/L)</u>							
Autumn newcomer <sup>†</sup>	54.1 (23.0)	55.3 (28.9)	54.4 (26.0)	53.9 (38.0)			0.261 <sup>a</sup>
Asian, autumn newcomer <sup>†</sup>	47.6(25.5)	59.0(32.2)	58.4(29.4)	58.3(41.2)			0.442 <sup>a</sup>
Spring newcomer <sup>†</sup>			47.1 (19.5)	44.8 (18.7)	49.4 (30.2)	46.7 (18.4)	0.101 <sup>a</sup>
Asian, spring newcomer <sup>†</sup>			48.4 (13.0)	43.9 (13.4)	50.1 (29.6)	46.9 (17.5)	0.676 <sup>a</sup>
Resident <sup>†</sup>		43.6 (18.8)	42.7 (22.1)	40.7 (24.0)	41.4 (16.0)		0.854 <sup>a</sup>
Asian, resident <sup>†</sup>		41.4 (20.6)	42.6 (18.9)	40.0 (22.2)	40.1 (15.6)		0.752 <sup>a</sup>

<sup>†</sup>Median, IQR, <sup>‡</sup>Mean, SD. <sup>a</sup>: Repeated measures ANOVA using natural log-transformed data

Table 4: Mixed model analysis to identify predictors of vitamin D status (Model A)

Independent variable	Estimate	SEM	p	95% CI		
				Lower bound	Upper bound	
<b><i>Dependent variable ln total 25(OH)D</i></b>						
Intercept	4.19	0.24	<0.001	3.72	4.67	
Ethnicity	Middle Eastern (n=8)	-0.42	0.13	0.002	-0.67	-0.16
	African (n=10)	-0.82	0.22	<0.001	-1.25	-0.39
	Caucasian/Hispanic (n=5)	0.80	0.17	<0.001	0.46	1.13
	Asian (n=48, ref)	0				
Season	Spring	0.05	0.04	0.201	-0.03	0.12
	Summer	0.23	0.05	<0.001	0.15	0.32
	Autumn	0.17	0.04	<0.001	0.10	0.25
	Winter (ref)	0				
Holiday abroad (yes vs no)	0.10	0.03	0.004	0.03	0.16	
Age (year)	-0.01	0.01	0.028	-0.03	-0.002	
ITA inner arm (°)	-0.01	0.003	<0.001	-0.02	-0.01	
Days in Scotland (no of days)	-0.0002	0.0001	0.001	-0.0003	-0.0001	
Total vitamin D intakes, including supplement (40 IU/day)	0.008	0.001	<0.001	0.004	0.012	

Ref: Reference; ITA: individual typology angle

Estimate: association between ln 25(OH)D concentration and independent variable

Using the equation from mixed model:  $\ln 25(\text{OH})\text{D} = 4.19 - 0.42(\text{Middle East}) - 0.82(\text{African}) + 0.80(\text{Caucasian/Hispanics}) + 0.05(\text{spring}) + 0.23(\text{summer}) + 0.17(\text{autumn}) + 0.10(\text{holiday in sunny location}) - 0.01(\text{age}) - 0.01(\text{ITA inner arm}) - 0.0002(\text{number of days in Scotland}) + 0.008(\text{for 40 IU/day total vitamin D intakes})$  predicts that a male Asian aged 30 years with 365 days in Scotland, never had holiday in sunny location, ITA of 28.4° (inner arm) and total vitamin D intake of 150 IU/day would have ln 25(OH)D of 3.56 in winter, which is equivalent to 35.3 nmol/L 25(OH)D (backtransformed by anti-logging the natural logarithm).

Table 5: Mixed model analysis to identify predictors of hand grip (Model B) and isometric knee strength (Model C to Model E)

Independent variable		Estimate	SEM	p	95% CI	
					Lower bound	Upper bound
<b><u>Model B: Dependent variable hand grip strength</u></b>						
Constant		-59.80	15.07	<0.001	-89.91	-29.69
Ethnicity	Middle Eastern	-0.06	1.68	0.701	-4.01	2.71
	African	6.80	1.62	<0.001	3.56	10.04
	Caucasian/Hispanic	1.44	2.24	0.522	-3.03	5.92
	Asian (ref)	0				
Sex (female vs male)		-8.96	1.43	<0.001	-11.82	-6.10
Height (cm)		0.54	0.09	<0.001	0.36	0.72
Weight (kg)		0.09	0.03	0.008	0.02	0.16
25(OH)D (10 nmol/L)		0.04	0.1	0.682	-0.2	0.3
<b><u>Model C: Dependent variable ln isometric knee strength</u></b>						
Constant		2.151	0.547	<0.001	1.059	3.243
Sex (female vs male)		-0.173	0.054	0.002	-0.282	-0.065
Height (cm)		0.015	0.003	<0.001	0.009	0.022
Weight (kg)		0.003	0.002	0.035	0.0002	0.006
25(OH)D (10 nmol/L)		0.014	0.01	0.047	0.0002	0.03
<b><u>Model D: Dependent variable ln isometric knee strength, with the inclusion of VDBP</u></b>						
Constant		2.09	0.58	0.001	0.92	3.26
Sex (female vs male)		-0.17	0.06	0.004	-0.28	-0.06
Height (cm)		0.01	0.004	<0.001	0.01	0.02
Weight (kg)		0.003	0.002	0.094	-0.001	0.01
25(OH)D (10 nmol/L)		0.01	0.01	0.049	0.0001	0.03
VDBP (μmol/L)		0.03	0.02	0.083	-0.004	0.06
<b><u>Model E: Dependent variable ln isometric knee strength, with the inclusion of BioD</u></b>						
Constant		2.01	0.56	0.001	0.89	3.12
Sex (female vs male)		-0.16	0.05	0.004	-0.27	-0.05
Height (cm)		0.02	0.003	<0.001	0.01	0.02
Weight (kg)		0.003	0.002	0.072	-0.0003	0.01
BioD (μmol/L)		0.01	0.01	0.277	-0.005	0.02

Ref: Reference; 25(OH)D: 25-hydroxyvitamin D; VDBP: vitamin D binding protein; BioD: bioavailable 25(OH)D

Estimate: association between hand grip strength or ln isometric knee strength and independent variable

The equation for Model C ln isokinetic knee strength = 2.151 -0.173(female) +0.015(height) +0.003(weight) +0.014(for 10 nmol/L 25(OH)D) predicts that a male Asian who is 172 cm height and 70 kg weight with 35 nmol/L 25(OH)D would have ln isometric knee strength of 4.99, which is equivalent to 147 Nm.

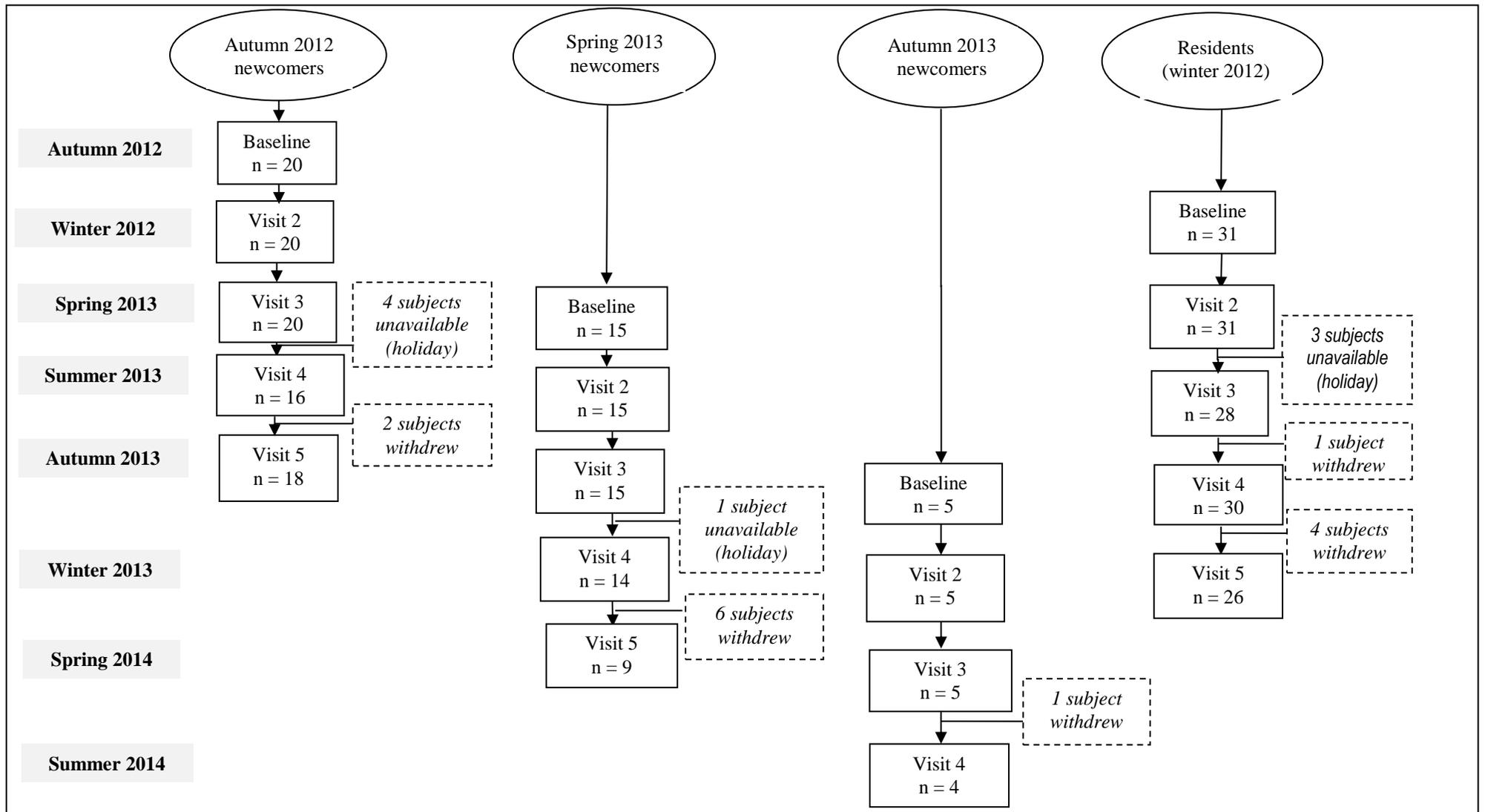


Figure 1: Participant's enrolment and retention

