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► To cite this version:

Anna-Maija Tolppanen, Dylan Williams, John Henderson, Da Lawlor. Serum 25-hydroxy-vitamin D and ionised calcium in relation to lung function and allergen skin tests. *European Journal of Clinical Nutrition*, 2011, 10.1038/ejcn.2011.6 . hal-00614737

HAL Id: hal-00614737

<https://hal.science/hal-00614737>

Submitted on 16 Aug 2011

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1 **Serum 25-hydroxy-vitamin D and ionised calcium in relation to lung function and**
2 **allergen skin tests**

3

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19 **Running title:** Vitamin D, calcium, lung function and atopy

20

21 **KEYWORDS**

22 vitamin D, calcium, allergy, spirometry, the third National Health and Nutrition Examination

23 Survey

24

25 Word count: main text 3354, abstract 249

1 **ABSTRACT**

2 **Background:** Evidence suggests that higher levels of vitamin D and calcium are associated
3 with greater lung function and that vitamin D is inversely associated with atopic sensitisation.
4 It is unknown whether the associations of vitamin D and calcium with lung function are
5 independent of each other or mediated by atopic sensitisation.

6 **Objective:** To study the associations of 25-hydroxyvitamin D [25(OH)D] and ionised
7 calcium levels with lung function and **specific** allergen sensitisation in adolescents (12-19
8 years) and adults (20-59 years) and to assess whether the associations with lung function are
9 due to altered atopic sensitisation.

10 **Methods:** Cross-sectional analysis of the data from the third National Health and Nutrition
11 Examination Survey.

12 **Results:** 25(OH)D levels were positively associated with forced vital capacity in adolescents
13 [0.035(95 %CI: 0.007-0.064) standard deviations;SD **in model adjusted for multiple**
14 **confounders**]. This association and the previously reported association between higher serum
15 levels of 25(OH)D and better lung function in adults were independent of serum calcium
16 levels, which were not associated with lung function. In adults, calcium was associated with
17 sensitisation to grass allergens [OR per SD, 1.17(1.03-1.32), 1.15(1.01-1.31) and 1.18 (1.06-
18 1.32) for white oak, Bermuda grass and short ragweed, respectively] and peanut OR 1.21
19 (95%CI 1.02-1.43) after adjusting for age, gender and race/ethnicity, but these associations
20 attenuated towards the null after adjusting for additional confounders. The associations were
21 independent of 25(OH)D levels, which were not associated with allergen sensitisation.

22 **Conclusions:** Circulating levels of 25(OH)D are positively associated with lung function and
23 this does not appear to be driven by allergen sensitisation or influenced by calcium levels.

24

25

1 INTRODUCTION

2

3 Atopy is characterised by a tendency to mount immunoglobulin E (IgE)-mediated, type I
4 hypersensitivity reactions to allergens. It is strongly associated with allergic diseases,
5 including asthma. In many Western countries 10-20 percent of the population are affected by
6 atopy and the prevalence of atopic diseases in children and adults has increased in these
7 countries from the 1970s (Asher et al., 2006; Upton et al., 2000). Although there is evidence
8 of genetic heritability (Swarr and Hakonarson, 2010), little is known about modifiable risk
9 factors for atopy that might be used in prevention.

10

11 Vitamin D is a key regulator of calcium homeostasis, and biological evidence suggests that
12 vitamin D and calcium might be implicated in atopic diseases and respiratory health,
13 (Hughes and Norton, 2009; Litonjua, 2009; Hirota et al., 2007; Chhabra et al., 1999) though
14 epidemiological evidence is limited and inconsistent (Devereux et al., 2007; Laaksi et al.,
15 2007; Ginde et al., 2009; Erkkola et al., 2009; Kunisaki et al., 2010; Hypponen et al., 2004;
16 Hypponen et al., 2009; Searing et al., 2010; Miyake et al., 2010). Differences in how vitamin
17 D exposure and outcomes were assessed or differences in the confounders that were taken
18 into account in different studies may have contributed to differences between them.

19 Wheezing and associated reduced lung function are amongst the wide range of symptoms
20 associated with atopy and there is some evidence of positive association between vitamin D
21 (measured as serum 25-hydroxyvitamin D [25(OH)D] and lung function (Devereux et al.,
22 2007; Searing et al., 2010; Brehm et al., 2009; Black and Scragg, 2005; Sutherland et al.,
23 2010), although a null association has also been reported (Devereux et al., 2009).

24

1 Previous studies in the US third National Health and Nutrition Examination Survey
2 (NHANES III) have shown positive association between serum calcium and forced
3 expiratory volume in one second (FEV₁) (McKeever et al., 2008) but not with atopy
4 (McKeever et al., 2004). In addition, higher serum 25(OH)D levels were associated with
5 better lung function in the same study (Black and Scragg, 2005), but it is unknown whether
6 the associations of 25(OH)D were independent of calcium and *vice versa*.

7
8 Our study adds to these previous papers by: (i) examining whether the associations of
9 25(OH)D and calcium are independent of each other: (ii) examining the association of
10 25(OH)D and calcium with **specific** allergen sensitisation: (iii) studying the association
11 between serum calcium and FVC and (iv) comparing whether the magnitudes and/or
12 directions of these associations differ between adolescents and adults. The previous lung
13 function studies have included participants older than 17 years only (McKeever et al., 2008)
14 or older than 20 years (Black and Scragg, 2005). The association between calcium and **d** atopy
15 **was** studied in 6-16 year olds and 17-59 year olds (McKeever et al., 2004).

18 **METHODS**

20 **Sample**

21 Data from the third US NHANES III were used. NHANES III, conducted between 1988 and
22 1994, is a survey of the civilian non-institutionalized population of the United States. Data
23 and documentation are available at <http://www.cdc.gov/nchs/nhanes/nh3rrm.htm>. Serum
24 25(OH)D and calcium measurements were available for participants aged ≥ 12 years and skin
25 prick allergen responses for participants up to age 59 years (with a random 50 percent of 20-

1 59 year olds having these tests). Thus, we included participants aged 12-59 years, examining
2 adolescents (12-19 years) and adults (20-59 years) separately. Figure 1 shows the flow of
3 participants who attended mobile examination clinics and final analyses samples for the
4 studies in adolescents and adults. We only included individuals with complete data on
5 exposures, outcome and all covariables in any analyses meaning that the final sample sizes
6 were 2446 and 2315 adolescents for associations with lung function and allergy tests,
7 respectively and 8049 and 3656 adults for associations with lung function and allergy tests,
8 respectively. The NHANES III study was approved by NHANES Institutional Review Board
9 and documented consent was obtained from participants.

10

11 **Exposures**

12 Serum levels of 25(OH)D and calcium were measured on venous samples. 25(OH)D was
13 assayed with INCSTAR 25-OH-D I²⁵ radioimmunoassay (Diasorin, Stillwater, MN, USA)
14 and serum calcium with NOVA 7 analyzer (Nova Biomedical, Waltham, MA, USA). Ionised
15 calcium (adjusted for serum pH) was used in these analyses.

16

17 **Outcomes**

18 Lung function and allergy tests were conducted at mobile examination centres (MECs). Lung
19 function [FEV₁ and forced vital capacity (FVC)] was assessed with a Ohio 827 rolling seal
20 spirometer (Ohio Medical Instrument Company, Cincinnati, OH USA) according to
21 American Thoracic Society criteria (1987) (American Thoracic Society, 1987).

22

23 The allergen panel consisted of 10 common allergens (*Dermatophagoides farinae*, cat,
24 German cockroach, short ragweed, perennial rye, *Alternaria alternata*, Bermuda grass,
25 Russian thistle, white oak and peanut) and a negative (glycerinated diluent) control.

1 Hypersensitivity reactions were evaluated 15 minutes after administering the allergens on
2 separate sites of examinee's forearms with prick-puncture technique. A positive test response
3 was defined as maximum weal diameter ≥ 3 mm for allergen and < 3 mm for negative control.
4 Atopy was defined as a positive response to one or more allergens. Individuals with severe
5 eczema or signs of skin infection on both arms, those with previous severe response to
6 allergen testing or participants with signs of breathing difficulties were excluded from the
7 allergy tests. We appreciate that these might include individuals with most severe atopy, but
8 due to small numbers (39 adolescents; 111 adults) it was not possible to examine this as an
9 outcome by itself in both adolescents and adults.

10

11 **Potential confounders**

12 The following were considered potential confounding factors due to their known or plausible
13 associations with exposures and outcomes: age, gender, race/ethnicity, height, health status,
14 socioeconomic position, outdoor activity and smoking.

15

16 Height and weight were measured using standard research procedures at the MECs and were
17 used to calculate body mass index (BMI). Physicians' impressions of health status
18 (Poor/Fair/Good/Very good/Excellent) at the MECs were used as an indicator of general
19 health. Data on self-reported race/ethnicity, smoking, pets, socioeconomic background,
20 outdoor physical activity and history of respiratory/allergic diseases (emphysema, chronic
21 bronchitis, asthma and hay fever) were obtained during interview. Family poverty-income
22 ratio (below/equal to or above 1) was used as indicator of socioeconomic position. Outdoor
23 activity during past month for adults was derived from the interview data according to
24 method by Scragg and Camargo (Scragg and Camargo, Jr., 2008). These data were not
25 collected from those aged 18 or younger. In adolescents, we adjusted for pubertal stage,

1 which was assessed by Tanner scales (Tanner JM, 1962). Among adults, smoking status was
2 defined as never, former (has smoked ≥ 100 cigarettes but not currently smoking), and current
3 smoker. Pack-years of smoking were calculated using answers to 12 tobacco questions and
4 included assessment of current number of cigarettes smoked, maximum number of cigarettes
5 smoked, and periods during which a current smoker did not smoke. Due to the skewed
6 distribution, a categorical variable for pack-years was derived (0, 0.1-4.9, 5-9.9, 10-19.9 and
7 ≥ 20 pack-years). Because of missing data on pack-years (22% of the adults), in our main
8 analyses we adjusted for smoking status only and in sensitivity analyses we examined the
9 effect of adjusting for pack-years in those with these data. In adolescents, smoking status was
10 classified as current smoker or non-smoker currently due to low frequency of ex-smokers
11 (n=67, 6.3% of those with smoking data available). Because 61.7% of adolescents did not
12 answer the smoking questionnaire, we did not include smoking as a covariable in the main
13 analyses in adolescents but conducted a sensitivity analysis in those with smoking data
14 available to see if adjusting for smoking affected the associations.

15

16 **Statistical analyses**

17 Statistical analyses were conducted with Stata 11.0 (Stata Corp LP, College Station, TX
18 USA), using the svy procedure to account for cluster effects and sampling weights from
19 MECs (lung function) and allergy subsamples (allergen sensitisation). A more detailed
20 description of recommended statistical methodology is available in (Landis et al., 1982). The
21 associations of 25(OH)D and calcium levels with lung function were analyzed with
22 multivariable linear regression. The association with atopy and sensitisation to allergens were
23 assessed with multivariable logistic regression. Serum calcium, 25(OH)D and spirometry
24 measurements were analyzed as z-scores.

25

1 RESULTS

2

3 The weighted mean (standard error) 25(OH)D and ionised serum calcium levels among
4 adolescents were 80.3 (1.80) nmol/l and 1.25 (0.003) mmol/l. Among adults, the weighted
5 mean 25(OH)D was 75.0 (1.00) nmol/l and weighted mean calcium level was 1.24 (0.003)
6 mmol/l. The correlation coefficient r for serum 25(OH)D and ionised calcium was 0.02
7 ($P=0.34$) in adolescents and 0.04 ($P=0.0009$) in adults. There was no statistical evidence that
8 any associations differed between males and females (P for interaction ≥ 0.13). Therefore,
9 results are presented for males and females combined.

10

11 Supplementary Tables 1-4 summarise the age- and gender-adjusted characteristics of
12 adolescents and adults across quartiles of serum 25(OH)D and ionised calcium. The
13 proportion of females, non-Hispanic blacks and persons from households with
14 poverty/income ratio below 1 decreased across the quartiles of 25(OH)D in adolescents,
15 while the proportion of non-Hispanic whites increased across the quartiles. Adolescents in the
16 higher quartiles had lower Tanner scores. Among adults, mean age and BMI decreased across
17 quartiles of 25(OH)D, and mean serum level of ionised calcium and height increased across
18 the quartiles. The proportion of men, non-Hispanic whites, those reporting outdoor physical
19 activity during past month, and those who had a pet increased across the quartiles of
20 25(OH)D. The proportion of non-Hispanic blacks, individuals with fair health status and
21 individuals from households with poverty-income ratio below 1 decreased across the
22 quartiles of 25(OH)D in adults.

23

24 The proportion of men increased linearly across the quartiles of ionised serum calcium in
25 adults. Mean age and number of pack-years smoked decreased across the calcium quartiles in

1 adults. Among the 12-19 year olds, mean BMI increased across the quartiles. In the adult
2 group, mean serum 25(OH)D increased across the quartiles, while the proportion of Mexican-
3 American and Other ethnicities decreased across the quartiles.

4

5 With respect to outcomes, in these age- and gender-adjusted models 25(OH)D was associated
6 positively with FEV₁ and FVC and inversely with responsiveness to German cockroach
7 allergen in both age groups. In adults, 25(OH)D was inversely associated with the prevalence
8 of atopy, and in adolescents, decreased responsiveness to ragweed, Bermuda grass and
9 perennial rye. Serum ionised calcium was positively associated with sensitisation to short
10 ragweed, Bermuda grass, white oak and peanut allergens in adults only. Ionised calcium was
11 not associated with any outcomes in adolescents, or with other outcomes in adults. Where
12 associations occurred they appeared linear across the quartiles.

13

14 Tables 1 and 2 show multivariable associations of 25(OH)D and calcium with lung function
15 in adolescents and adults respectively. In adolescents (Table 1), 25(OH)D was positively
16 associated with FVC in the confounder adjusted model, but not with FEV₁. Additional
17 adjustment for pubertal stage (n=1566) did not alter the associations presented (results
18 available from authors). In adults (Table 2) serum 25(OH)D levels were positively associated
19 with both FEV₁ and FVC after adjusting for all potential confounders (Model 2). Ionised
20 serum calcium was not associated lung function in adolescents or adults (Tables 1-2). With
21 additional mutual adjustment (associations of 25(OH)D adjusted for ionised calcium and *vice*
22 *versa*) the associations remained unchanged (Model 3 in Tables 1-2).

23

24 Tables 3 and 4 summarise the multivariable associations of 25(OH)D and calcium levels with
25 previously diagnosed respiratory or allergic diseases and atopic outcomes in adolescents and

1 adults. No associations were observed in adolescents (Table 3) and serum 25(OH)D levels
2 were not associated with allergic diseases or allergen sensitisation in adults, apart from
3 slightly decreased sensitisation to German cockroach allergen (Table4), Higher calcium
4 levels were associated with increased sensitisation to short ragweed, Bermuda grass, white
5 oak and peanut allergens, when adjusted for age, gender, race/ethnicity and height (model 1).
6 With adjustment for additional confounders (Model 2) and 25(OH)D (Model3) the
7 associations with sensitisation to short ragweed and Bermuda grass allergen remained, but
8 other associations were attenuated to the null.

9
10 Sensitivity analyses are shown in supplementary Tables 5-12. In the subgroups of
11 adolescents with smoking data and adults with data on pack-years associations that were the
12 equivalent of Models 1-3 for the whole eligible sample (Tables 1-4) were markedly different
13 in inconsistent ways, suggesting that analyses in these subgroups may be affected by
14 selection bias. Adjusting for smoking in adolescents and pack-years in adults did not
15 markedly alter associations in these subgroups.

16

17 **DISCUSSION**

18

19 We showed that the previously reported positive association of 25(OH)D with FVC and
20 FEV₁ in adults (Black and Scragg, 2005) is independent of serum calcium levels, and not due
21 decreased risk of allergen sensitisation or respiratory diseases, as serum 25(OH)D levels were
22 not associated with these outcomes. In addition, the association between 25(OH)D levels and
23 FVC, but not with FEV₁ was observed in adolescents, whereas a previous publication using
24 NHANES III data had only explored this association in adults (Black and Scragg, 2005). As
25 with adults, this association was independent of calcium and allergen sensitisation. We did

1 not find an association between ionised serum calcium and lung function in either adolescents
2 or adults, although a previous study reported a positive association between ionised calcium
3 and FEV₁ among NHANES III participants age ≥ 17 years (McKeever et al., 2008). That
4 previous study did not address the association between FVC and calcium. The difference
5 between our results and those of that previous NHANES study are likely to be due to
6 differences in age ranges and numbers of participants included in the analyses of the two
7 papers. We were interested in exploring whether associations differed between adolescents
8 (defined as those aged 12-19) and adults, who had data on allergen sensitisation (20-59 year
9 old). By contrast McKeever *et al.* were exploring associations in adults only (defined as
10 anyone aged 17 years or older), consequently they had larger numbers. McKeever et al. also
11 categorised calcium in a different way to the way we have used this variable. When we repeat
12 our analyses using calcium in the same way as that used by McKeever et al. we still find no
13 strong statistical evidence of an association in either of the age groups we examined, by
14 contrast we can perfectly replicate McKeevers results in those aged 17 years and above.

15

16 **Vitamin D and lung function**

17 Several mechanisms may link higher vitamin D levels to better lung function. The positive
18 association of 25(OH)D with lung function might be the result of vitamin D protecting
19 against atopic asthma, but we found no evidence that higher levels of 25(OH)D were
20 associated with decreased allergic responsiveness to common allergens. FEV₁ is primarily
21 used as a measure of intrathoracic airway obstruction, as seen in asthma, in clinical and
22 epidemiological settings, although changes in total lung capacity (TLC) will affect both FEV₁
23 and FVC. FVC is a dynamic representation of the difference between TLC and residual
24 volume and, in healthy subjects, it is likely to vary with TLC and reflect lung growth. It is
25 therefore possible that the positive association of 25(OH)D with FVC in adolescents reflects

1 the established role of vitamin D in skeletal growth (Holick, 2005), i.e. higher 25(OH)D is
2 related to greater height via beneficial effects on skeletal development and greater height is
3 associated with greater lung volume. However, all associations were adjusted for height and
4 so this is unlikely to fully explain the association. In adults 25(OH)D was positively
5 associated with both FEV₁ and FVC, supporting a role beyond the relation to skeletal
6 development.

7

8 High consumption of antioxidants have been associated with lung function,(e.g (Gilliland et
9 al., 2003)) so it is possible that the association of vitamin D is mediated via its antioxidative
10 properties. Alternative mechanisms might be the regulation of immune response, cellular
11 differentiation and/or proliferation by vitamin D (Bikle, 2009; van Etten et al., 2008).

12 Vitamin D receptor is present in human bronchial smooth muscle cells, epithelial cells lining
13 the respiratory tract, activated lymphocytes and antigen-presenting cells(van Etten et al.,
14 2008; Bosse et al., 2007), which support the local functions of vitamin D in the lungs and in
15 the immune system. Therefore, it could be that vitamin D regulates the growth of bronchial
16 smooth muscle cells or protects from the lung inflammation by promoting the expression of
17 antimicrobial peptides and through anti-inflammatory mechanisms (Bikle, 2009; van Etten et
18 al., 2008). Alterations in calcium homeostasis are known to occur in asthma sufferers
19 (Chhabra et al., 1999), so it would be tempting to speculate this as a possible mechanism,
20 although in our study circulating levels of ionised calcium were not associated with lung
21 function, However, despite the lack of association between serum calcium levels and lung
22 function, local calcaemic effects such as alterations in intracellular calcium signalling could
23 still relate to the mechanism.

24

25 **Ionised calcium and atopy**

1 Our results suggest that variation in ionised serum calcium is more robustly associated with
2 allergic response to common allergens in adults than is variation in vitamin D. In adults
3 positive associations were found with sensitisation to grass allergens and peanut, although
4 apart from short ragweed and Bermuda grass, these associations were attenuated towards the
5 null after adjusting for confounders. Experimental studies suggest that calcium is involved in
6 allergic response: bronchial smooth muscle contraction, mast cells granulation and histamine
7 release from mast cells are calcium-dependent (Hirota et al., 2007; Alm, 1984) and effects of
8 vitamin D on the immune system depend on adequate calcium levels (Cantorna et al., 1999).
9 Both intra- and extracellular calcium levels have been shown to regulate the histamine release
10 (Alm, 1984), so one would perhaps expect to see a disruption of calcium homeostasis in
11 atopy, although intracellular calcium levels are strictly controlled and thus unlikely to be
12 affected by normal variation in serum calcium levels. In addition, the calcium-binding motifs
13 in allergens, especially of grass origin, are necessary for IgE binding and the chelation of
14 calcium ions from sera of allergic patients lead to decreased allergen-IgE binding or
15 prevented this occurring (Suphioglu et al., 1997; Ledesma et al., 2002). To our knowledge
16 only one previous study has examined the association of circulating calcium with allergic
17 response and that cross-sectional study found no association (McKeever et al., 2004). The
18 positive associations we have found here in adults require further exploration.

19

20 **Study strengths and limitations**

21 The main strengths of this study are its sample size, examination of associations with serum
22 measurements of 25(OH)D and calcium rather than dietary reports, examination with
23 objectively assessed outcomes of lung function and allergic response to common allergens,
24 comparison of associations in adolescents and adults and the ability to adjust for a wide range
25 of potential confounding factors. In adults we were only able to adjust for smoking status

1 (current/ex/never) for our main analyses and, whilst sensitivity analyses suggested that
2 adjustment for pack-years in the subgroup with these data did not result in greater attenuation
3 compared with smoking status, there was some evidence that selection bias may have
4 influenced this subgroup analysis. Similarly, in adolescents we were unable to adjust for
5 smoking in the main analysis and those with smoking data appeared to be a selected
6 subgroup.

7

8 Because this study is cross-sectional, we cannot rule out reverse causality as an explanation
9 for the observed associations. Further exploration in prospective studies are warranted,
10 though we are unaware of any studies that currently have measurements of serum 25(OH)D
11 and calcium with later objectively measured lung function and response to common
12 allergens. NHANES III has single measurements of calcium and 25(OH)D, which may be
13 inadequate to reflect long-term status (Schram et al., 2007), although a single measurement
14 may be a useful biomarker of season-specific vitamin D status over a longer time (Hofmann
15 et al., 2010) and serum calcium levels are normally maintained at relatively narrow limits
16 within individuals (Parfitt, 1987). Other studies, including those finding associations with
17 bone phenotypes (Jesudason et al., 2002), have used single measurements of 25(OH)D. A
18 further limitation is the lack of data on IgE and parathyroid hormone levels.

19

20 **Conclusions**

21 Our findings contribute to the emerging literature on a possible role of vitamin D and calcium
22 with lung function and atopy. We showed that the positive cross-sectional association
23 between serum 25(OH)D levels and lung function is independent of variation in ionised
24 calcium levels and does not appear to be driven by increased allergen sensitisation. By
25 contrast we report a novel positive association between serum levels of ionised calcium and

1 grass allergen sensitisation in adults, which is independent of a wide-range of potential
2 confounding factors and of vitamin D.

3

4 Supplementary information is available at The European Journal of Clinical Nutrition's
5 website.

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1 **FUNDING**

2 Work on this study is funded by an UK Medical Research Council (MRC) Grant
3 (G0701603), which also pays AMT's salary. The MRC and the University of Bristol provide
4 core funding for the MRC Centre of Causal Analyses in Translational Epidemiology
5 (G0600705). DW is funded by a Wellcome Trust 4-year PhD studentship in Molecular,
6 genetic and lifecourse epidemiology [WT083431MA].

7

8 The views expressed in this paper are those of the authors and not necessarily those of any
9 funding body or others whose support is acknowledged. The funders had no role in study
10 design, data collection and analysis, decision to publish, or preparation of the manuscript.

11

12 **COMPETING INTERESTS**

13 None

14

15 **ABBREVIATIONS**

16 25(OH)D: 25-hydroxyvitamin D

17 BMI: body mass index

18 CI: confidence interval

19 FEV₁: forced expiratory volume in one second

20 FVC: forced vital capacity

21 IgE: immunoglobulin E

22 MEC: mobile examination center

23 NHANES III: the third National Health and Nutrition Examination Survey

24 SD: standard deviation

25 TLC total lung capacity

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2 **Figure legends**

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4 **Figure 1.** Flowchart of adolescent and adult participants

1 **Table 1.** Association of circulating 25(OH)D and ionised calcium (n2446) with lung function in 12-19 year olds.

		Mean difference in respiratory function (SD) per 1 SD change in 25(OH)D (95%CI)			Mean difference in respiratory function (SD) per 1 SD change in ionised serum calcium (95%CI)		
	SD	Model 1 ¹	Model2 ²	Model3 ³	Model 1 ¹	Model2 ²	Model3 ³
FEV ₁	753	0.009 (-0.028 to 0.046)	0.009 (-0.028 to 0.046)	0.008 (-0.028 to 0.045)	0.010 (-0.024 to 0.045)	0.010 (-0.024 to 0.045)	0.010 (-0.024 to 0.044)
FVC	901	0.036 (0.007 to 0.065)	0.036 (0.007 to 0.065)	0.035 (0.007 to 0.064)	0.007 (-0.024 to 0.038)	0.007 (-0.024 to 0.038)	0.005 (-0.025 to 0.034)

2 FEV₁ forced expiratory volume in 1 second; FVC forced vital capacity

3 ¹Model 1 is adjusted for age, ethnicity/race, gender and height

4 ²Model 2 as Model 1, plus additional adjustment for BMI, poverty index, household pets (cat/dog/bird), general health and diagnosed
5 asthma/hay fever/chronic bronchitis

6 ³Model 3 as Model 2, plus additional adjustment for calcium in 25(OH)D analysis and 25(OH)D in calcium analysis

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1 **Table 2.** Association of circulating 25(OH)D and ionised calcium(n=8049) with lung function in 20-59 year olds.

		Mean difference in respiratory function(SD) per 1 SD change in 25(OH)D(95%CI)			Mean difference in respiratory function(SD) per 1 SD change in ionised serum calcium(95%CI)			
		SD	Model 1 ¹	Model2 ²	Model3 ³	Model 1 ¹	Model2 ²	Model3 ³
FEV ₁	821		0.034 (0.016 to 0.052)	0.023 (0.004 to 0.041)	0.023 (0.005 to 0.041)	0.006 (-0.012 to 0.025)	0.002 (-0.018 to 0.021)	0.000 (-0.019 to 0.019)
FVC	1006		0.043 (0.024 to 0.061)	0.032 (0.015 to 0.049)	0.032 (0.015 to 0.049)	0.004 (-0.018 to 0.025)	-0.001 (-0.022 to 0.020)	-0.003 (-0.024 to 0.018)

2 FEV₁ forced expiratory volume in 1 second; FVC forced vital capacity

3 ¹Model 1 is adjusted for age, ethnicity/race, gender and height

4 ²Model 2 as Model 1, plus additional adjustment for BMI, poverty index, household pets(cat/dog/bird), general health, outdoor activity during
5 last month, smoking and diagnosed asthma/emphysema/hay fever/chronic bronchitis

6 ³Model 3 as Model 2, plus additional adjustment for calcium in 25(OH)D analysis and 25(OH)D in calcium analysis

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2 **Table 3.** Association of circulating 25(OH)D and ionised calcium levels with previously diagnosed respiratory/allergic diseases (asthma, chronic
3 bronchitis or hay fever) (n=2446) and allergy test results (n=2317) in 12-19 year olds

	Odds ratio for each allergen outcome per 1SD change in 25(OH)D (95%CI)			Odds ratio for each allergen outcome per 1SD change in ionised serum calcium (95%CI)			
	Prev (%)	Model 1 ¹	Model2 ²	Model3 ³	Model 1 ¹	Model2 ²	Model3 ³
Respiratory disease	16.3	0.92 (0.76-1.13)	0.92 (0.76-1.13)	0.92 (0.75-1.12)	1.09 (0.91-1.29)	1.09 (0.91-1.29)	1.09 (0.92-1.30)
Atopy	60.6	1.05 (0.89-1.24)	1.06 (0.90-1.26)	1.06 (0.90-1.26)	1.03 (0.89-1.19)	1.01 (0.88-1.17)	1.01 (0.87-1.16)
<i>D.farinae</i>	31.1	0.96 (0.79-1.18)	0.97 (0.80-1.17)	0.97 (0.80-1.18)	0.98 (0.83-1.15)	0.96 (0.81-1.14)	0.96 (0.81-1.14)
Cat	18.4	0.93 (0.78-1.10)	0.93 (0.78-1.11)	0.94 (0.78-1.12)	0.94 (0.76-1.18)	0.93 (0.73-1.18)	0.93 (0.73-1.18)
German cockroach	32.9	0.90 (0.77-1.05)	0.92 (0.79-1.08)	0.92 (0.79-1.08)	1.03 (0.85-1.26)	1.03 (0.83-1.27)	1.03 (0.84-1.27)
Short ragweed	31.6	0.92 (0.79-1.06)	0.94 (0.81-1.08)	0.93 (0.81-1.08)	1.06 (0.91-1.23)	1.04 (0.90-1.22)	1.05 (0.90-1.22)

Bermuda grass	23.1	0.95 (0.80-1.14)	0.96 (0.79-1.16)	0.96 (0.79-1.16)	0.98 (0.80-1.21)	0.97 (0.79-1.20)	0.98 (0.79-1.20)
Perennial rye	31.6	0.96 (0.81-1.14)	0.96 (0.81-1.14)	0.96 (0.82-1.14)	1.00 (0.87-1.14)	0.99 (0.86-1.14)	0.99 (0.86-1.14)
Russian thistle	18.6	1.06 (0.90-1.25)	1.08 (0.91-1.29)	1.08 (0.91-1.30)	0.95 (0.78-1.16)	0.94 (0.76-1.17)	0.94 (0.75-1.18)
White oak	13.5	0.99 (0.83-1.18)	1.01 (0.83-1.24)	1.01 (0.83-1.24)	1.03 (0.80-1.33)	1.02 (0.76-1.38)	1.02 (0.76-1.38)
<i>A. alternata</i>	18.2	1.06 (0.89-1.26)	1.09 (0.90-1.32)	1.09 (0.90-1.32)	1.08 (0.86-1.35)	1.07 (0.83-1.38)	1.06 (0.82-1.38)
Peanut	10.6	0.92 (0.74-1.15)	0.93 (0.74-1.17)	0.93 (0.74-1.17)	0.99 (0.82-1.21)	0.98 (0.81-1.18)	0.98 (0.81-1.19)

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2 Prev: Prevalence

3 ¹Model 1 is adjusted for age, ethnicity/race, gender and height4 ²Model 2 as Model 1, plus additional adjustment for BMI, poverty index, household pets (cat/dog/bird), general health and (for other outcomes
5 than respiratory disease) diagnosed asthma/hay fever/chronic bronchitis6 ³Model 3 as Model 2, plus additional adjustment for calcium in 25(OH)D analysis and 25(OH)D in calcium analysis

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2 **Table 4.** Association of circulating 25(OH)D and ionised calcium levels with previously diagnosed respiratory/allergic diseases(asthma, chronic
3 bronchitis or hay fever, n=8049) and allergy test results (n=3656) in 20-59 year olds

	Odds ratio for each allergen outcome per 1SD change in 25(OH)D (95%CI)			Odds ratio for each allergen outcome per 1SD change in ionised serum calcium (95%CI)			
	Prev (%)	Model 1 ¹	Model2 ²	Model3 ³	Model 1 ¹	Model2 ²	Model3 ³
Respiratory disease	16.1	0.96 (0.85-1.09)	0.96 (0.85-1.09)	0.96 (0.84-1.09)	1.04 (0.95-1.15)	1.04 (0.94-1.15)	1.04 (0.94-1.16)
Atopy	57.8	0.97 (0.85-1.11)	0.98 (0.86-1.12)	0.98 (0.85-1.12)	1.06 (0.97-1.15)	1.03 (0.95-1.13)	1.03 (0.95-1.13)
<i>D.farinae</i>	29.0	1.01 (0.88-1.16)	1.02 (0.89-1.18)	1.02 (0.89-1.17)	1.04 (0.93-1.17)	1.03 (0.91-1.16)	1.03 (0.92-1.15)
Cat	18.1	1.07 (0.91-1.27)	1.08 (0.91-1.27)	1.07 (0.91-1.27)	1.09 (0.98-1.22)	1.04 (0.92-1.16)	1.03 (0.92-1.16)
German cockroach	32.3	0.89 (0.79-1.01)	0.89 (0.79-1.01)	0.89 (0.78-1.01)	1.05 (0.95-1.15)	1.04 (0.94-1.15)	1.05 (0.95-1.16)
Short ragweed	29.2	1.04 (0.91-1.19)	1.05 (0.92-1.21)	1.05 (0.91-1.20)	1.15 (1.01-1.31)	1.14 (1.01-1.29)	1.15 (1.02-1.30)
Bermuda grass	20.8	0.96 (0.83-1.10)	0.95 (0.82-1.10)	0.94 (0.81-1.09)	1.18 (1.06-1.32)	1.14 (1.01-1.29)	1.15 (1.02-1.30)

Perennial rye	28.5	0.98 (0.87-1.12)	0.97 (0.85-1.12)	0.97 (0.85-1.12)	1.06 (0.97-1.16)	1.02 (0.94-1.12)	1.03 (0.94-1.12)
Russian thistle	17.7	0.92 (0.80-1.07)	0.91 (0.78-1.06)	0.91 (0.78-1.06)	1.10 (0.96-1.27)	1.06 (0.91-1.24)	1.07 (0.91-1.24)
White oak	13.5	1.03 (0.88-1.21)	1.02 (0.87-1.20)	1.02 (0.87-1.19)	1.17 (1.03-1.32)	1.11 (0.98-1.26)	1.11 (0.98-1.26)
<i>A. alternata</i>	13.5	1.04 (0.90-1.21)	1.03 (0.87-1.21)	1.03 (0.88-1.22)	0.99 (0.86-1.15)	0.94 (0.81-1.08)	0.93 (0.81-1.08)
Peanut	9.8	1.01 (0.86-1.18)	1.00 (0.86-1.16)	0.99 (0.85-1.16)	1.19 (1.02-1.39)	1.15 (0.98-1.34)	1.15 (0.98-1.34)

1 Prev: Prevalence

2 ¹Model 1 is adjusted for age, ethnicity/race, gender and height

3 ²Model 2 as Model 1 plus additional adjustment for BMI, poverty index, household pets (cat/dog/bird), general health, outdoor activity during
4 last month, smoking and (for other outcomes than respiratory disease) diagnosed asthma/emphysema/hay fever/chronic bronchitis

5 ³Model 3 as Model 2, plus additional adjustment for calcium in 25(OH)D analysis and 25(OH)D in calcium analysis

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